Proceedings of the Indian Academy of Sciences
(Plant Sciences)

Volume 98, 1988 9

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Status of *Brachiolejeunea* (Spruce) Schiffn. in India*

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MS received 22 January 1987; revised 16 November 1987

Abstract. The status of Indian *Brachiolejeunea* (Spruce) Schiffn. is discussed. The species described earlier under this genus belong to closely allied genera *Frullanoides* Raddi or *Trocholejeunea* Schiffn. *Frullanoides* Raddi is known only by *Frullanoides tristis* (Steph.) Van Slageren which has been discovered recently from Nilgiri hills (Tamil Nadu) and Nandi hills (Karnataka) in southern India. *Trocholejeunea* is represented by two species, *Trocholejeunea infuscata* (Mitt.) Verd. from eastern Himalayas and *Trocholejeunea sandvicensis* (Gott.) Mizut. from Palni hills (Tamil Nadu), south India and western Himalaya. Of these *Trocholejeunea sandvicensis* has been discovered for the first time from western Himalaya which also shows intracapsular spore germination of the *Lopholejeunea*-type. The scanning electron microscopic details of sporoderm of *Frullanoides tristis* and *Trocholejeunea sandvicensis* have been given.

Keywords. Bryophyta; Jungermanniales; Lejeuneaceae; *Brachiolejeunea*; *Frullanoides*; *Trocholejeunea*.

1. Introduction

The first Indian species of *Brachiolejeunea* (Spruce) Schiffn. sensu lato was described by Mitten (1861) as *Lejeunea infuscata* Mitt. from Sikkim and Khasi hills. It was later transferred to *Trocholejeunea* by Verdoorn (1934). Later, Stephani (1912–1917) described 3 species—*B. andamana* St. from Andaman, *B. wardiana* (Mitt.). St. from Himalaya and *B. levieri* Schiffn. from Sikkim Himalaya. Schiffner (1932) described *B. levieri* as *Trocholejeunea levieri* (St.) Schiffn. from Darjeeling and Kurseong. Verdoorn (1934) rightly treated *B. andamana* and *B. wardiana* as synonyms of *Ptychanthus striatus* (Lehm. et Lindenb.) Nees and *Mastigolejeunea repleta* (Tayl.) St., respectively, while *B. levieri* was treated under *Trocholejeunea infuscata* (Mitt.) Verd. Under the genus *Brachiolejeunea*, he described *B. sandvicensis* (Gott.) Evs. from Perumalmalai, Palni hills (see also Mizutani 1961). Herzog (1939), however, retained *B. levieri* and reported it from Darjeeling. He also described *B. aliculata* Herz. from Shembaganur (Herzog 1950) which seems to be the synonym of *B. sandvicensis*. Mizutani (1978) transferred this species (*B. sandvicensis*) to *Trocholejeunea* Schiffn.

It is thus evident that *Brachiolejeunea* and *Trocholejeunea* have been controversial genera for a long time. Schuster (1980) pertinently remarked "... it is difficult, if not impossible, to maintain the distinction between *Brachiolejeunea* and *Trocholejeunea* accepted by Verdoorn (1934) and, following him, Gradstein (1975) and others". Considering intrageneric diversity within *Brachiolejeunea* sensu lato, Schuster (1980) divided *Brachiolejeunea* into 3 subgenera—*Brachiolejeunea* (Spruce) Schiffn., *Plicolejeunea* Schust. and *Trocholejeunea* (Schiffn.) Schust., the last with sections *Pluriplicatae*, *Trocholejeunea* and *Sandvicensis*. Van Slageren (1985) raised the subgenus *Plicolejeunea* to the generic rank as *Frullanoides* Raddi, and considered th other two subgenera also as distinct genera. According to him the 3 genera differ a shown in table 1.

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*Contribution New Series (Bryophyta) No. 211.

Table 1 evidently shows that *Brachiolejeunea* is distinctly different from the othe two genera particularly in sporophytic characters and convincingly merits a generi status. However, *Frullanoides* and *Trocholejeunea* are closely related as they do n possess sufficiently contrasting stable characters and therefore subject to controvers till more information in this regard is provided. But as the generic delimitation i Ptychanthoideae is difficult for many taxa due to variability and plasticity in variou characters, the generic status could be drawn only after judging the over all feature and taking them into consideration we agree with Van Slageren's (1985) treatme which has been adopted in the present communication.

In view of the above treatment (Van Slageren 1985) the genus *Brachiolejeunea* altogether lacking in India and *Trocholejeunea* is represented by *T. infuscata* (Mitt Verd. and *T. sandvicensis* (Gott.) Mizut. and *Frullanoides* by *F. tristis* (Steph.) Va Slageren, the latter recently discovered from Nilgiri hills (Tamil Nadu) and Nan hills (Karnataka), south India. *T. sandvicensis*, earlier known from Palni hills (sout India) has an extended range in the Himalaya. Intracapsular spore germination ha been described for the first time in *T. sandvicensis*. The scanning electron microscopi (SEM) details of sporoderm pattern for Indian plants of *F. tristis* and *T. sandvicens* have also been provided.

Table 1.

Characters	*Brachiolejeunea*	*Frullanoides*	*Trocholejeunea*
Pigmentation	without secondary pigments	with secondary pigments	without secondary pigments
Stem	cortex thick-walled and medulla thin-walled or outer medulla and cortex thickened and inner medulla thin-walled	medulla thick-walled cortex thin-walled	cortex and medulla more or less equally thick-walled
Dorsal merophyte	in straight line	zig-zag	in straight line
Branching *Frullania*- or *Lejeunea*-type	*Frullania*-type	Both *Frullania* and *Lejeunea*-type	Both *Frullania* a *Lejeunea*-type
Leaf-lobule	1–4 toothed	3–11 toothed	2–4 toothed
Underleaf attachment	tristratose	bistratose	bistratose
Perianth	with 0–1 (–2) ventral plicae	with 2–5 ventral plicae	with 2–5 ventral plicae
Subfloral innovation	*Jubula*-type	*Jubula*-type	*Frullania*-type
Seta	articulate	articulate	not articulate
Seta in cross-section	16+4 celled	16+4 celled	(16–32)+(8–16) cell
Valve of the capsule at dehiscence	suberect	curving outwardly	curving outwardly
Inner layer of capsule wall	nodular	fenestrate	fenestrate
Spores	irregularly elongated	isodiametric	isodiametric
Elaters	with wall thickenings only	with well developed spiral	with well develop spiral
Sporeling	*Lejeunea*-type	*Lopholejeunea*-type	*Lopholejeunea*-type

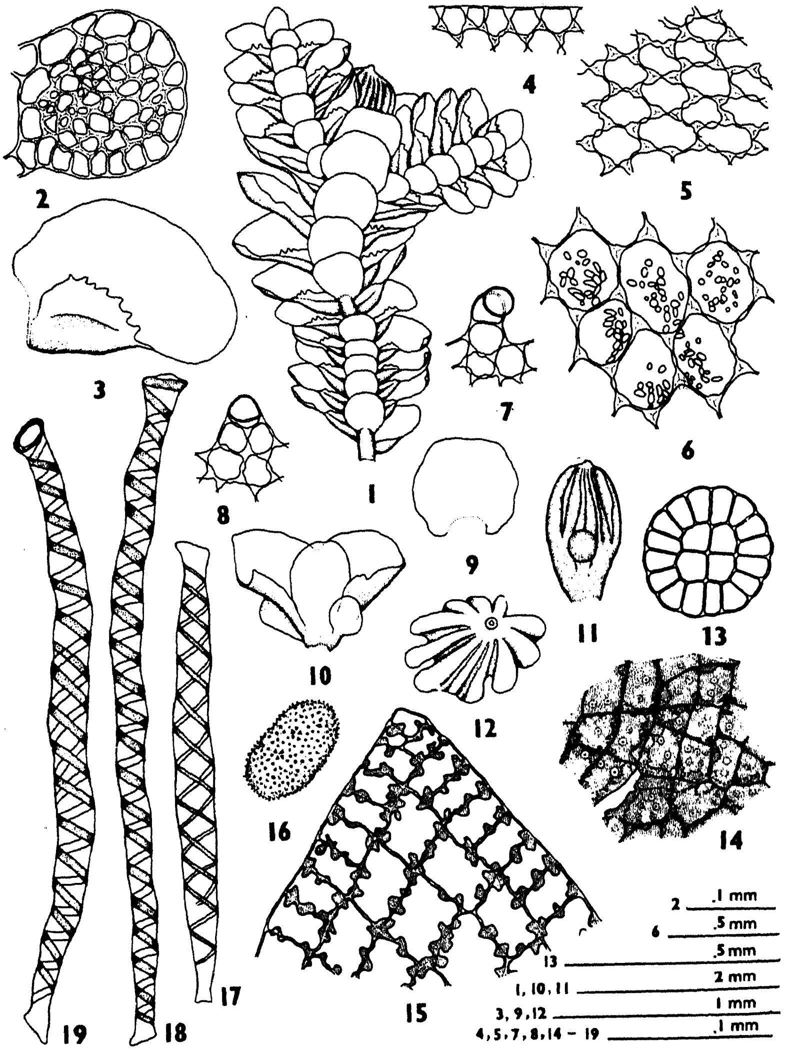
2. Taxonomic description

2.1 *Frullanoides Raddi*

2.1a *Frullanoides tristis* (Steph.) Van Slageren Meded Bot. Mus. Herb. Van. de Rij te Utrecht 544: 110 (1985). *Lejeunea* (subgen. *Brachiolejeunea*) *tristis* Steph. Hedwigia 29 (1): 8, 29 (3): 134 (1890) *Brachiolejeunea tristis* (Steph.) Steph. Spec. Hepat. 5: 112 (1912): Arnel (1961: 400; 1963; 225), Vanden Berghen (1951: 88; 1960: 114). *Brachiolejeunea poeltii* Mizut. and Grolle, Ergebn. Forsch. Untern. Nepal Himalaya 1 (4): 293 (1966) (figures 1–19, 59, 60).

Plants brownish in colour, irregularly branched by *Lejeunea*-type or *Frullania-Ptychanthus*-type (first leafy appendage) on a branch is a bilobed underleaf of branching. Stem in cross-section (0·15–0·20 mm in diameter) with 18–20 cortical cells and 35–40 slightly smaller medullary cells, both cortical and medullary cells thick-walled with distinct trigones. Leaves imbricated, widely spreading, slightly squarrose, ovate, ovate-oblong, 0·85–1·00 mm long, 0·51–0·69 mm wide, entire-wavy at margin, antical margin convex, postical margin slightly incurved at about middle, also often revoluted along with apex, apex rounded, obtuse, or rarely subacute, cells arranged in diverging rows, with distinct cordate trigones and intermediate nodular thickenings, basal cells 24–36 × 24–28 μm, median cells 20–32 × 16–20 μm, marginal cells 12–20 × 12–16 μm, lobule ovate 1/2 or slightly more than 1/2 of the lobe length, 0·30–0·51 mm long, 0·27–0·33 mm wide, with 4–7 marginal teeth of 2–3 cells in height and 1–2 cells in width. Underleaves imbricated, slightly auriculate at base with usually arched or rarely sinuate insertion, slightly longer than wide, or as long as wide, 0·48–0·51 mm long, 0·41–0·48 mm wide, margin entire-wavy, apex rounded, rhizoids rare. Female inflorescence terminal on an elongated branch with two subfloral innovations of *Radula-Jubula*-type (in which first appendage on the branch is underleaf) bractlobe ovate or oblong, 1·12–1·47 mm long, 0·64–1·00 mm wide, margin entire-wavy, apex subacute or rounded, lobule almost rectangular more than 1/2 of the lobe length, 0·40–0·67 mm long, 0·16–0·24 mm wide, adnate with the lobe or slightly extending beyond the keel, keels with variously shaped appendages; bracteole almost 1/2 of the perianth length, somewhat rectangular, 0·83–0·89 mm long, 0·57–0·59 mm wide, with entire-wavy margin, apex rounded or truncate; perianth oblong, ca 1·85 mm long, 0·70 mm wide, isoplicate, plicae 10, wide and smooth. Seta in cross-section with 16 peripheral and 4 central cells. Capsule wall bistratose, outer layer with sinuate-nodular thickenings, inner layer with fenestrate thickenings on inner tangential walls and sinuate thickenings on radial wall. Spores green, variously shaped, 48–60 μm in diameter or 60–80 × 44–52 μm, ornamented with small papillae and few 'rosettes' of spines. Elaters trumpet shaped, 400–480 mm long, 16–24 mm wide, with 1 (–2) spiral thickening bands.

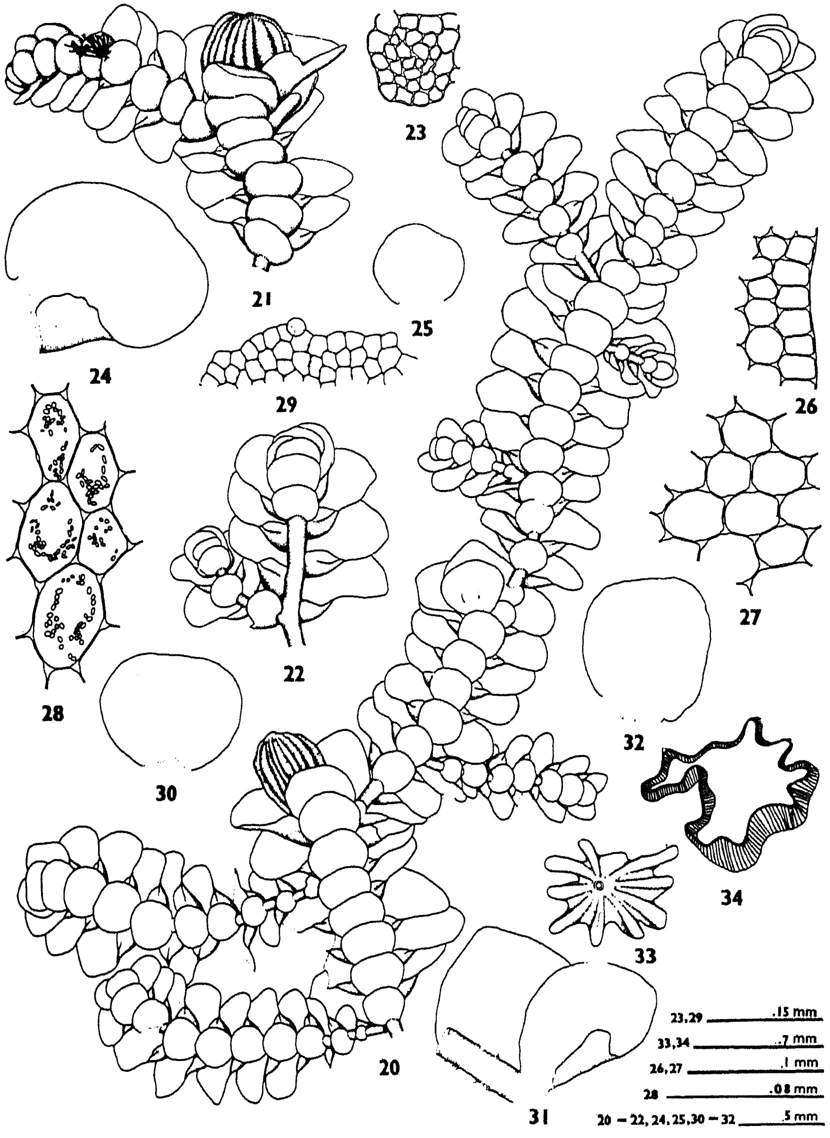
2.1b *Specimens from India examined*: LWU No. 128 SE/72, Loc.: Near Naduvattam (Mysore road), Nilgiri hills (alt. ca 1982 m), Tamil Nadu, south India, Dt.: January 3, 1972, Leg.: R Udar and party. LWU No. 7101/83, Loc.: Mukurty, Ootacamund (alt. 2500 m), Tamil Nadu, south India, Dt.: September 27, 1983, Leg.: R Udar and party. LWU No. 7501/83, 7523/83, Loc.: Near Kodai lake, Kodaikanal (alt. 2500 m), Tamil Nadu, south India, Dt.: September 30, 1983, Leg.: R Udar and party. LWU No. 7539/83, Loc.: Shembaganur, Kodaikanal (alt. 2500 m), Tamil



Figures 1–19. **1–12.** *Frullanoides tristis* (Steph.) Van Slageren **1.** Portion of the plant showing female inflorescence with two subfloral innovation. **2.** Cross-section of the stem. **3.** Leaf. **4.** Marginal cells of the leaf-lobe. **5.** Median cells of the leaf-lobe. **6.** Median cells of the leaf-lobe with oil-bodies. **7 and 8.** Marginal teeth of the leaf-lobule. **9.** Underleaf. **10.** Female bracts and bracteole. **11.** Perianth. **12.** Apex of the perianth. **13.** Cross-section of the seta. **14.** Inner layer of the capsule wall. **15.** Outer layer of the capsule wall. **16.** Spore. **17–19.** Elaters.

Nadu, south India, Dt.: September 30, 1983, Leg.: R Udar and party. LWU No. 7972/84, Loc.: Nandi hills, Bangalore, Dt.: June 5, 1984, Leg.: U S Awasthi and A K Asthana.

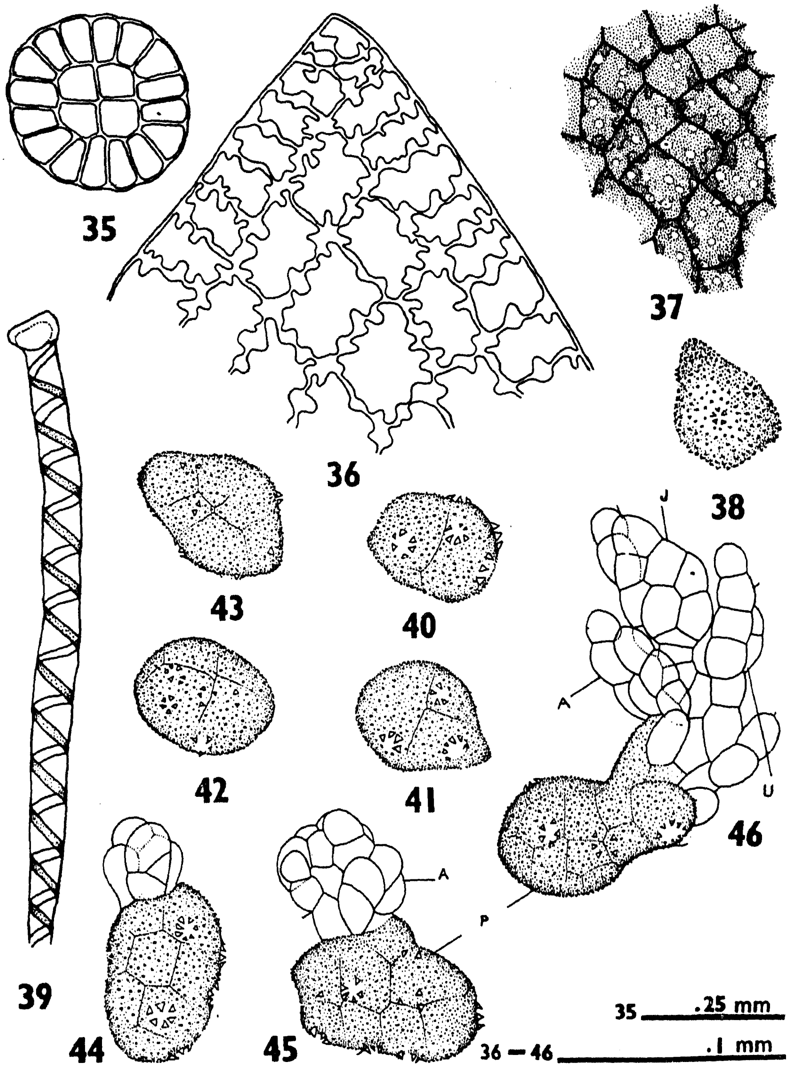
2.1a *Specimens from other countries examined*: JE H208/8, NICH 242111 (Isotype) *Brachiolejeunea poeltii* Mizut. et Grolle. Loc.: Nepal, Leg.: Poelt, Dt.: 1962, Det.: Mizut. and Grolle, G 739 Type *Frullanoides tristis* (St.) Van Slageren (*Brachiolejeunea*



Figures 20–34. *Trocholejeunea sandvicensis* (Gott.) Mizut. **20 and 21.** Portion of the plant showing female inflorescences. **22.** Portion of the plant showing *Lejeunea*-type branch. **23.** Cross-section of the stem. **24.** Leaf. **25.** Leaf subtending the *Frullania*-type branch. **26.** Marginal cells of the leaf-lobe. **27.** Median cells of the leaf-lobe. **28.** Basal cells of the leaf-lobe with oil-bodies. **29.** Portion of leaf-lobule showing marginal teeth. **30.** Underleaf. **31.** Female bracts with subfloral innovation. **32.** Female bracteole. **33.** Apex of the perianth. **34.** Cross-section of perianth.

tristis (St.) St. Loc.: Ethiopia (hb. Lindenberg 5996), Det. M Van Slageren. G 740 *Frullanoides tristis* (St.) Van Slageren, Loc.: Kilimanjaro, Tanzania, Leg.: Hans Meyer, Dt.: 1890, Det.: M Van Slageren.

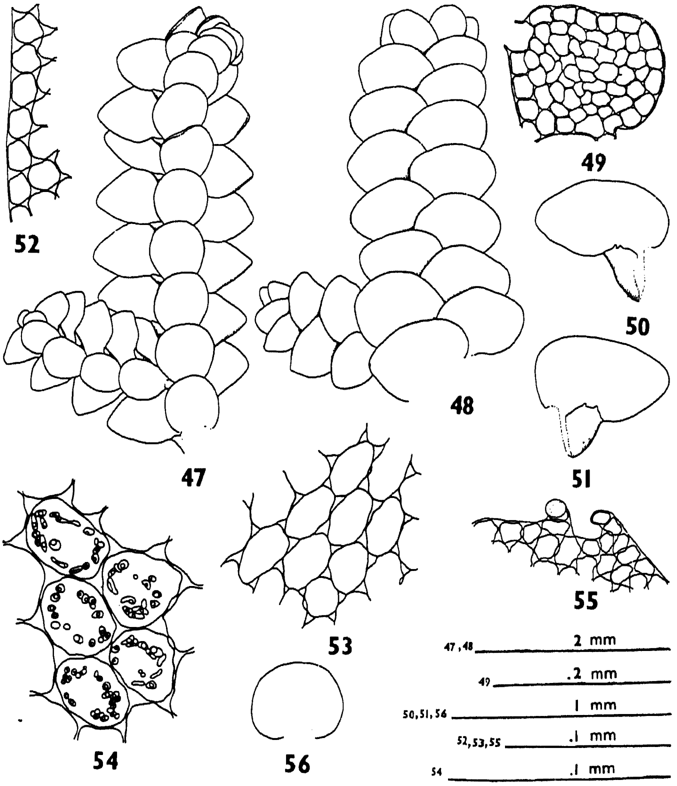
2.1d *Distribution*: India (Tamil Nadu), Angola, Brazil, Burundi, Colombia,



Figures 35–46. *Trocholejeunea sandvicensis* (Gott.) Mizut. **35.** Cross-section of the seta. **36.** Outer layer of the capsule wall. **37.** Inner layer of the capsule wall. **38.** Spore. **39.** Elater. **40–46.** Stages of spore germination.
(P, Protonema; L, primary leaf; J, juvenile leaf; U, underleaf).

Cameroun, Costa Rica, Cuba, Ethiopia, Gålapagos Islands, French Guiana Honduas, Jamaica, Madagascar, Nepal, Nigeria, Peru, Panama, Puerto Rico, Rewanda, South Africa, Sierra Leone, Tanzania, Venezuela and Zaire.

2.1e *Discussion*: In *Frullanoides tristis* obtained from different herbaria, the leaves generally have acute or subacute apices. However, in Indian plants they are mostly obtuse or rounded (figures 1, 3). Van Slageren (1985) described the apex varying from rounded to blunt to (occasional) minutely apiculate in this taxon. His consideration of *Brachiolejeunea poeltii* Mizut. et Grolle as synonym of *F. tristis* seems reasonable.



Figures 47–56. *Trocholejeunea infuscata* (Mitt.) Verd. **47 and 48.** Portion of the plant. **49.** Cross-section of the stem. **50 and 51.** Leaves. **52.** Marginal cells of the leaf. **53.** Median cells. **54.** Median cells with oil-bodies. **55.** Portion of the leaf-lobule showing marginal teeth. **56.** Underleaf.

2.2 *Trocholejeunea* Schiffn.

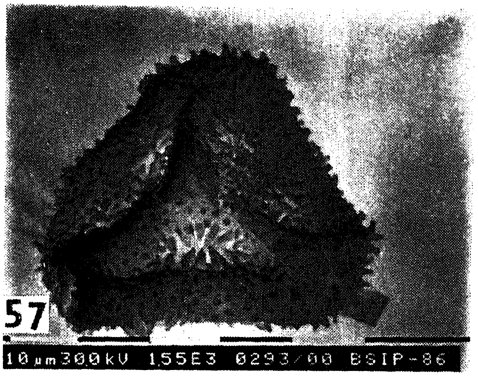
2.2a *Key to the Indian species of Trocholejeunea*:

1. Leaf-lobe with postical margin straight, long axis of lobule parallel with the long axis of lobe, lobular teeth 2 (–3) in number . . . . . *T. sandvicensis.*

1. Leaf-lobe with postical margin curved or bowed, long axis of lobule almost at right angle to the long axis of lobe, with 2 lobular teeth . . . *T. infuscata.*

2.2b *Trocholejeunea sandvicensis* (Gott.) Mizut. Misc. Bryol. Lichenol. 2: 169 (1962). *Brachiolejeunea sandvicensis* (Gott.) Evs. Trans. Conn. Acad. 10: 419 (1900). Mizutani (1961): 167–168. *Brachiolejeunea* (*Trocholejeunea*) *sandvicensis* (Gott.) Schust. Trans Conn. Acad. 10: 419 (1900), *Phragmicoma sandvicensis* Got. Ann. Sci. Nat. Ser. 4, 8: 344 (1857). *Brachiolejeunea aliculata* Herz. Mem. Soc. Faun. Fl. Fennica 26: 50 (1950) (figures 20–46, 57, 58).

Plants green in colour, irregularly branched by *Frullania-Ptychanthus* or *Lejeunea*-type of branching. Stem in cross-section (0·10–0·15 mm in diameter) with 12–13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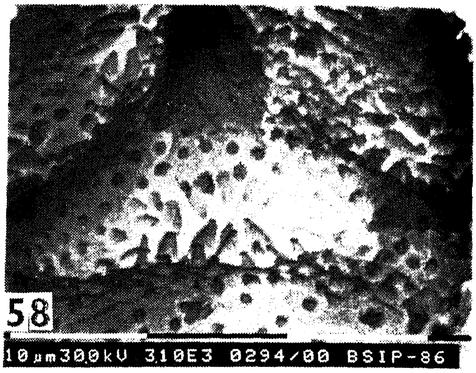
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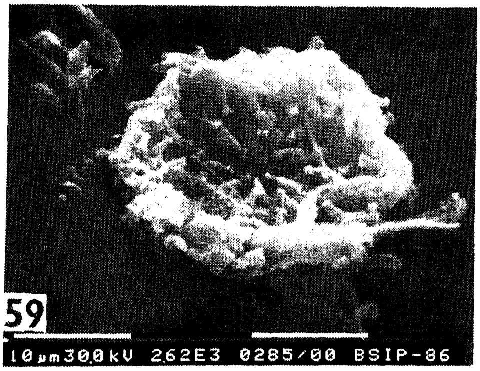
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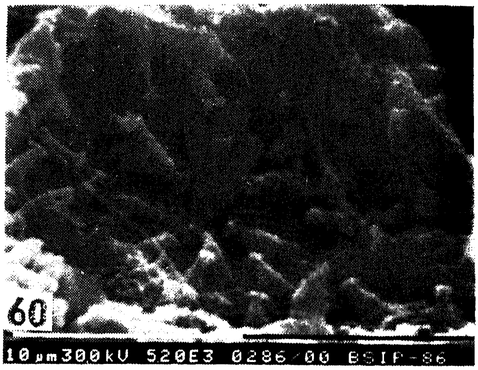
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Figures 57–60. 57 and 58. *T. sandvicensis.* **59 and 60.** *F. tristis.*

cortical and 15–16 slightly smaller medullary cells, both cortical and medullary cells almost thin-walled with inconspicuous trigones. Leaves usually imbricated widely spreading, sometimes slightly squarrose, lobe 0·82–1·04 mm long, 0·59–0·85 mm wide, ovate with entire margin, antical margin convex, postical margin almost straight, apex rounded, cells arranged in diverging rows, with distinct cordate trigones and intermediate nodular thickenings, basal cells 24–52 × 20–32 µm, median cells 28–44 × 24–36 µm, marginal cells 20–28 × 12–20 µm; oil-bodies several in each cells at middle and base of the lobe, rounded (1–2 µm in diameter) oval, elliptical or rod-shaped (2–4 × 1 µm), homogeneous, lobule ovate, slightly less than 1/2 of the lobe length, 0·35–0·40 mm long, 0·25–0·32 mm wide, ovate, ovate-quadrate, with 2 (–3) marginal teeth of the cell in height. Underleaves imbricated, wider than long, 0·41–0·56 mm long, 0·48–0·73 mm wide, with entire margin and rounded apex, rhizoids present at the base. Female inflorescence terminal on an elongated branch, usually with one subfloral innovation of the *Radula-Jubula*-type, bract-lobe almost equal to leaf-lobe in size, 0·72–1·13 mm long, 0·51–0·97 mm wide, ovate or obovate, dimorphic (one bract without lobule), margin entire, apex obtuse-rounded; bracteole larger than underleaf, slightly less than the length of the perianth, 0·72–0·74 mm long, 0·61–0·64 mm wide; perianth 0·80–1·0 mm long, 0·60–0·80 mm wide, 10 plicate, plicae unequal wide and smooth. Seta in cross-section with 16 peripheral and 12 central cells. Capsule wall bistratose cells of the outer layer with sinuate-nodular thickenings, cells of the inner layer with fenestrate thickenings on inner tangential wall and sinuate-nodular thickenings on radial walls. Spores with minute papillae and (rosettes' of spines, germination *in-situ*, *Lopholejeunea*-type, protonema few-celled, primary leaves ovate. Elaters trumpet shaped 400–800 mm long, 16–24 mm wide, with 1 (–2) spiral thickening bands.

2.2c *Specimens from India examined*: LWU No. 5197/81, Loc.: Naini Tal (alt. ca 2000 m), Dt.: October 1981, Leg.: S C Srivastava. LWU No. 6742/82, Loc.: Chaubatia forest, Ranikhet (alt. ca 2000 m), Dt.: November 10, 1982, Leg.: R Udar and U S Awasthi. LWU No. 7431/83, Loc.: Perumalmalai, Kodaikanal (alt. ca 2000 m), Dt.: September 29, 1983, Leg.: R Udar and party.

2.2d *Specimens from other countries examined*: JE 182a, *Brachiolejeunea sandvicensis*, Loc.: Koolan mts. Oalm (alt. 1800 ft), Dt.: January 12, 1930, Leg.: Edwin B Bartram, Det.: Fr Verdoorn. JE 333 *B. sandvicensis*. Loc.: Near Pahala Kau lo, Hawaii (alt. 500 ft), Dt.: January 30, 1930, Leg.: Edwin B Bartram, Det.: ?. JE 3730, *B. sandvicensis*, Loc.: Hawaii, Dt.:?, Leg.: Baldaii, Det.: Stephani, JE 12, *B. sandvicensis*, Loc.: Fundort: Tsingtan, Dt.: August 6, 1945, Leg.:?, Det.:?. JE 26 *B. sandvicensis*, Loc.: Tring-Henrich-Berg, Tsingtan, China, Leg.: S H Schwabe. JE 52, *B. sandvicensis*, Loc.: Formosa, Dt.: January 29, 1947, Leg.: Schwabe-Behn, Det.:?. JE 2 *B. sandvicensis*, Loc.: Formosa, Dt.: June 21, 1976, Leg.: Schwabe-Behn, Det.:?. JE 9 *B. sandvicensis*, Loc.: Formosa, Dt.:?. Leg.: Schwabe, Det.:?. JE 95 *B. sandvicensis*, Loc.: Formosa, Dt.: June 4, 1947, Leg.: Schwabe-Behn JE 11405 *B. sandvicensis*, Loc.: Hunan, Dt.: December 2, 1917, Leg.: H F V Handel-Mazzetti, Det.: Herzog. JE 10982, *B. sandvicensis*, Loc.: Kweitschou orient. (alt. 750 m), Dt.: July 25, 1917, Leg.: H F V Handel-Mazzetti, Det.: Herzog. JE 11512 *B. sandvicensis*, Loc.: Hunan (alt. 100 m), Dt.: March 8, 1918. Leg.: H F V Handel-Mazzetti, Det.: Herzog. JE 12163 *B. sandvicensis*, Loc.: Hunan (alt. 1200 m), Det.: June 1918, Leg.: H F V Handel-Mazzetti. JE 11433 *B. sandvicensis*, Loc.: Hunan, Dt.: December 10, 1917, Leg.: H F V Handel-Mazzetti, Det.: Herzog. JE 11889 *B. sandvicensis*, Loc.: Hunan (alt. 550 m), Dt.: May 20, 1918, Leg.: H F V Handel-Mazzetti, Det.: Herzog. JE 451, *B. sandvicensis*, Loc.: Yunnan, Dt.: March 9, 1914, Leg.: H F V Handel-Mazzetti. JE 8 *B. sandvicensis*, Loc.: Formosa, Dt.: September 29, 1946, Leg.: Schwabe-Bebu. Det.:?. JE 6651 *Trocholejeunea sandvicensis*, Loc.: Ouyamada-mura (alt. 400 m), Dt.: May 31, 1964, Leg.: K Yamada, Det.: K Yamada. JE 3211 *T. sandvicensis*, Loc.: Hawaii: Kailu, Keolu hills (alt. 100 ft), Dt.: March 10, 1977, Leg.: C W Smith. Det.:?. JE 640, *T. sandvicensis*, Loc.: Bhutan: Thimphu Chu (alt. 2600 m), Dt.: June 21, 1975, Leg.: A J C Grierson and D G Long, Det.: Grolle. JE 163 *T. sandvicensis*, Loc.: Bhutan: Paro chu Valley (alt. 2400 m), Dt.: June 10, 1975, Leg.: A J C Grierson and D G Long, JE 2699 *T. sandvicensis*, Loc.: Nepal: Mt. Phulchoki (alt. 1600–2300 m), Dt.: July 15, 1972, Leg.: Z Iwatsuki, Det.: Mizutani. JE 275, *T. sandvicensis*, Loc.: Mt. Kuniuni, Ohsumi, Japan, Dt.: February 17, 1974, Leg.: H Inoue, Det.: H Inoue.

2.2e *Distribution*: India (Tamil Nadu, Uttar Pradesh), Central Pacific, continental south eastern Asia, Hawaii, Japan, Sri Lanka and Taiwan.

2.2f *Discussion*: Some stages of *in-situ* spore germination (figures 40–46) answering to *Lopholejeunea*-type of spore germination (see Fulford 1956) have been observed. A few-celled protonema is first formed with in the exospore (figures 45, 46). The leafy shoot from this protonema bears ovate primary leaf (figures 45, 46), saccate inflated Juvenile leaf (figure 46) and narrow, ovate, underleaf (figure 46).

2.2g *Trocholejeunea infuscata* (Mitt.) Verd. Ann. Bryol. IV (Suppl.): 190 (1934). *Brachiolejeunea* (*Trocholejeunea*) *infuscata* (Mitt.) Schust. The Bryologist 64: 165 (1961). *Lejeunea infuscata* Mitt. J. Proc. Linn. Soc. (London) 5: 111 (1861). *Brachiolejeunea levieri* Schiffn. Spec. Hepat. 5: 134 (1912), *Trocholejeunea levieri* (Steph.) Schiffn. Ann. Bryol. 5: 160 (1932). *Ptychocoleus saccatus* (Mitt.) Steph. Spec. Hepat. 5: 53 (1912) (figures 47–56).

Plants brown, irregularly branched by *Frullania-Ptychanthus*-type of branching. Stem in cross-section (0·14–0·16 × 0·19–0·21 mm) with 18–20 (−23) cortical cells and 35–40, almost equal sized and less thickened medullary cells with inconspicuous trigones. Leaves imbricated, widely spreading, falcate, lobe 0·96–1·02 mm long, 0·64–0·69 mm wide, ovate with entire margin, antical margin convex, postical margin strongly incurved at the end of the keel, apex obtuse, often incurved, cells arranged in diverging rows distinct cordate trigones and intermediate nodular thickenings, basal cells 28–50 × 16–28 μm, median cells 32–40 × 20–28 μm, marginal cells 8–20 × 12–20 μm; oil-bodies several in each cell at middle and base, rounded (2·2–4·4 μm in diameter), oval or rod-shaped (4·4–11·0 × 1·1–2·2 μm) with refracting granule, homogeneous; lobule ovate, slightly less than 1/2 of the lobe length, 0·43–0·57 mm long, 0·25–0·27 mm wide, with two teeth of (1 −) 2–3 cells in height, often bend inwardly. Underleaves imbricated, slightly wider than long, 0·64–0·73 mm long, 0·68–0·81 mm wide, with entire but revoluted margin, apex rounded, rhizoids rare. Gynoecia and androecia not seen.

2.2h *Specimens from India examined*: LWU No. 3106/77, 3118A/77, Loc.: On way to Nathula pass, Sikkim (alt. ca 2,000 m), Dt.: December 28, 1977, Leg.: S C Srivastava, U S Awasthi and A Kumar. LWU No. 3233A/77, 3233B/77, Loc.: Kalimpong, West Bengal (alt. ca 1,000 m), Dt.: December 29, 1977, Leg.: S C Srivastava, U S Awasthi and A Kumar. LWU No. 3284A/77, Loc.: Llyod Botanic Garden, Darjeeling (alt. ca 2,000 m), Dt.: December 31, 1977, Leg.: S C Srivastava, U S Awasthi and A Kumar. LWU No. 5351/72, Loc.: Tiger hill, Darjeeling (alt. ca 2,500 m), Dt.: January 2, 1978, Leg.: S C Srivastava and party.

2.2i *Specimens from other countries examined*: G 15564 *Trocholejeunea infuscata* (Mitt.) Verd., Loc.: Kurseong, Sikkim, 1899, Leg.: Rev. Stevens. G 19970 (type) *Brachiolejeunea levieri* Schiffn. Loc.: Kurseong, Sikkim, Dt.: July 17, Rev. Pes Decoly et Schaul. G 19984 (type) *Homalolejeunea sikkimensis* St., Loc.: Sikkim, Dt.: 1898. Leg.?

2.2j *Distribution*: India (eastern Himalaya), Nepal, Sri Lanka.

2.2k *Discussion*: Plants of *T. infuscata* were collected repeatedly from several sites in eastern Himalaya but they were found only in sterile condition. Even the specimens (G 15564, G 19970 and G 19984) obtained on loan from conservatoire et Jardin Botanique were also sterile.

The leaf-lobule which lies almost at right angle to the leaf-lobe and consequently the postical margin of the leaf-lobe is not straight (figures 50, 51) in *T. infuscata* and thus differs from *T. sandvicensis* which has straight postical margin of the lobe (figure 24). Further, the lobule in *T. infuscata* has 2 teeth of (1 −) 2–3 cells in height (figure 55) while in *T. sandvicensis* it has 2–3 teeth of one cell in height (figure 29).

2.3 *SEM analysis of the spores* of *Fullanoides* and *Trocholejeunea spp.*

Earlier Udar and Awasthi (1983) described SEM details of spores of *Dicranolejeunea* (Spruce) Schiffn., *Schiffneriolejeunea* Verd. and *Spruceanthus* Verd. Van Slageren and Berendsen (1985) investigated the sporophytic features of Ptychanthoideae under SEM and depending on the sporoderm pattern categorised the taxa into 3 groups having capsule wall with fenestrate thickening on inner layer of cells.
1. Spinulate spore type (present in *Acrolejeunea*, *Frullanoides*, *Lopholejeunea Marchesinia*, *Ptychanthus*, *Schiffneriolejeunea Spruceanthus*, *Trocholejeunea* and *Thysananthus*).
2. Verrucate spore type (present in *Archilejeunea*)
3. Baculate-Spinulate spore type (present in *Caudalejeunea* and *Mastigolejeunea*).

The taxa having capsule wall with nodular type of thickening on inner layer of cells are highly variable with regard to sporoderm pattern which in this group serves to distinguish genera or even species.

The spores of *Frullanoides* and *Trocholejeunea* are spinulate type and thus more or less identical. However, the two species investigated (one of each genera): *F. tristis* and *T. sandvicensis* show some difference in the tip of the spine which is comparatively more fringed in *F. tristis* (figures 59, 60) and less so in *T. sandvicensis* (figures 57, 58).

2.4 *Current status of the Indian taxa described earlier*

1. *Brachiolejeunea andamana* St. Spec. Hepat. V: 130 (1912). =*Ptychanthus striatus* (Lehm. et Lindenb.) Nees Naturgesch Eur. Leberm. 3: 212 (1938).
2. *Brachiolejeunea aliculata* Herr. Mem. Soc. Faun. Fl. Fennica 26: 50 (1950). =*Trocholejeunea sandvicensis* (Gott.) Evs. Misc. Bryol. Lichenol. 2: 169 (1962).
3. *Brachiolejeunea levieri* Schiffn. Spec. Hepat. V: 134 (1912). =*Trocholejeunea infuscata* (Mitt.) Verd. Ann. Bryol. IV (Suppl.): 190 (1934).
4. *Brachiolejeunea sandvicensis* (Gott.) Evs. Trans. Conn. Acad. 10: 419 (1900). =*Trocholejeunea sandvicensis* (Gott.) Mizut. Misc. Bryol. Lichenol. 2: 169 (1962).
5. *Brachiolejeunea wardiana* (Mitt.) St. Spec. Hepat. V: 129 (1912). =*Mastigolejeunea repleta* (Tayl.) St. Spec. Hepat. IV: 772 (1912).
6. *Ptychocoleus saccatus* (Mitt.) St. Spec. Hepat. V: 53 (1912). =*Trocholejeunea infuscata* (Mitt.) Verd. Ann. Bryol. IV (Suppl): 190 (1934).
7. *Trocholejeunea levieri* (St.) Schiffn. Ann. Bryol. V: 160 (1932). =*Trocholejeunea infuscata* (Mitt.) Verd. Ann. Bryol. IV (Suppl.): 190 (1934).

Acknowledgements

Thanks are due to the late Professor Ram Udar for his interest and encouragement in the preparation of the present paper and to Drs S Hattori (Japan), Gilbert Bocquet (Geneva) and R Grolle (Jena) for kindly sending authentic specimens of *Brachiolejeunea*. The authors are grateful to Dr Hattori especially for critically going through the manuscript and providing helpful suggestions. Financial assistance is acknowledged from the Department of Science and Technology, University Grants Commission and Council of Scientific and Industrial Research, New Delhi.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 1, February 1988, pp. 13–23.
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Growth and allocation strategy of some important species of seral grasslands at Cherrapunji in north-eastern India

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MS received 7 May 1987; revised 3 December 1987

Abstract. Growth and allocation patterns of biomass and nutrients of herbaceous species from grasslands at Cherrapunji in north-eastern India were studied under varied nutrient stress situations. Though populations from a nutrient richer soil had better growth rate than those from nutrient poor sites, this difference was not reflected in the tissue concentration of nutrients. Populations in nutrient deficient soils had high allocation to the belowground organs of reproduction than for aboveground parts. Though *Ischaemum* and *Eragrostiella* had generally lower nitrogen uptake efficiencies, their use efficiency was fairly high. Soils that are generally nutrient deficient had more C_4 grass components than the rich soil under *Osbeckia*-type.

Keywords. Growth; resource allocation; nutrient; nutrient uptake efficiency; nutrient use efficiency.

1. Introduction

The grasslands at Cherrapunji, in Meghalaya in north-eastern India, one of the wettest places on Earth, represent various stages of arrested succession of the climax forest (Ram 1986). These are maintained at the grass stage because of climatic stress and is a consequence of clear-cutting the fragile forest ecosystem (Ramakrishnan *et al* 1981). The grasses and forbs of these degraded sites have ecophysiological attributes to survive in infertile soils subjected to frequent disturbance and climatic stress (Ramakrishnan 1985a, b).

Resource allocation strategy, both biomass and nutrients, of the species growing under such stress situations is a reflection of the fitness of the species for survival (Ramakrishnan 1984; Saxena and Ramakrishnan 1984). The present study attempts to look at the ecophysiological attributes of some of the important herbaceous species in grasslands under varied levels of nutrient stress at Cherrapunji.

2. Study area and climate

The study area at Cherrapunji (25°, 15′ N, 91°, 45′ E and 1313 m elevation) located 50 km south of Shillong is one of the wettest places of the world. The climate is monsoonal with an annual average rainfall of 10372 mm (mean for 1973–1983 period) about 96% of which occurs between April–October. The mean monthly maximum and minimum temperatures during this period are 24 and 14°C, respectively. November–February are winter months of which February is relatively a dry period. The mean monthly maximum and minimum for the winter period are 17 and 6°C, respectively. The dry summer is just for one transitional month between the winter and the rainy seasons, with mean monthly maximum and minimum temperatures of 20 and 12°C, respectively.

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3. Methods

Three grassland types, namely (i) *Osbeckia*–type, which is found in a relatively nutrient rich soil with an average soil profile depth of about 40 cm and which was burnt 6 years prior to the study period, (ii) *Ischaemum*–type, developed on a similar soil type but frequently burnt at 2-year intervals and (iii) *Eragrostiella*–type developed on a nutrient deficient soil at a highly desertified site, were selected.

Arundinella bengalensis, *Carex cruciata* and *Chrysopogon gryllus* are found as part of the *Osbeckia*–type on a nutrient rich soil, as well as on a nutrient poor desertified soil as components of the *Eragostiella*–type. *Ischaemum goeblii* is characteristic of frequently burnt, *Ischaemum*–type. *Eragrostiella leoptera* is found on nutrient poor desertified soils only. These 5 species are subsequently referred by their generic name only. The vegetation analysis of the 3 sites were based on 30 randomly placed quadrats of 1 m^2. Importance value indices (IVI), which is an integrated measure of the relative frequency, relative density and relative dominance (Curtis 1959), was calculated for each species.

Each species was harvested from 3 randomly placed quadrats of 50 × 50 cm at monthly intervals during the growing season. Harvested plants were separated into belowground (roots and rhizomes) and aboveground (stem, leaf and seed) components. Culm was considered as stem. Different components were dried at 80°C for 48 h and weighed. The biomass at sampling time was computed by also considering the number of fallen leaves as suggested by Hickman (1975). Leaf area was measured using a planimeter, and leaf dry weight per unit leaf area was based on 3 replicates, with 50 leaves per replicate. Total leaf area was computed from leaf dry weight per unit leaf area and leaf biomass value. Three growth functions, relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) (Hughes and Freeman 1967; Radford 1967) were calculated as:

$$\mathrm{RGR} = \frac{\mathrm{In}\, W_2 - \mathrm{In}\, W_1}{t_2 - t_1},$$

$$\mathrm{NAR} = \frac{(W_2 - W_1)(\mathrm{In}\, A_2 - \mathrm{In}\, A_1)}{(A_2 - A_1)(t_2 - t_1)},$$

$$\mathrm{LAR} = \frac{(A_2 - A_1)(\mathrm{In}\, W_2 - \mathrm{In}\, W_1)}{(\mathrm{In}\, A_2 - \mathrm{In}\, A_1)(t_2 - t_1)},$$

where W_1 and A_1 are biomass and leaf area at time t_1 and similarly W_2 and A_2, at time t_2.

Plant samples were analysed chemically following standard procedures given by

Allen *et al* (1974). Thus nitrogen was estimated by micro-kjeldahl method, phosphorus by molybdenum blue method and potassium by flame photometry after wet digestion with triple acid. Nutrient uptake efficiency was calculated as mg nutrient absorbed per g root biomass following Blair and Cordero (1978). Nutrient use efficiency was calculated as mg dry matter production per g nutrient absorbed (Brown 1978).

Ten random soil samples collected in July from the 0–10 cm horizon were pooled to give one composite sample for each fallow field. Soil was chemically analysed by standard methods (Jackson 1962; Allen *et al* 1974): organic carbon by Walkley-Black method, total nitrogen by kjeldahl method, nitrate-nitrogen by phenol-di-sulphonic acid method, available phosphorus by molybdenum blue method using Bray and Kurtz's extraction solution, and potassium by flame photometry after extraction in IM ammonium acetate solution at pH 7. All the analyses were replicated 3 times.

4. Results

Under *Osbeckia*–type, the species *A. bengalensis*, *C. cruciata*, *C. gryllus*, *Eulalia quadrinervis*, *Fimbristylis thomsonii* and *Osbeckia capitata* were abundant (table 1). After the burn, some species such as *Gentiana quadrifaria* and *I. goeblii* appeared, others such as *Phyllanthus simplex* increased in number, and yet others such as *Osbeckia crinita* declined. Under *Ischaemum*–type, species such as *A. bengalensis*, *I. goeblii*, *Fimbristylis complanata*, *Gentiana quadrifaria* and *O. capitata* were dominant. These species are also important components under *Eragrostiella* type, along with other species such as *E. leoptera* and *Borreria hispida*.

Table 1. IVI of species of grasslands at Cherrapunji in north-eastern India.

Species	*Osbeckia*–type	*Ischaemum*–type	*Eragrostiella*–type
Perennials			
Arundinella bengalensis (spreng) Druce.	24·6	14·6	12·1
Arundinella nepalensis Trin.	13·4	5·4	—
Carex cruciata Wahenb.	21·3	6·5	4·4
Chrysopogon gryllus (L.) Trin.	24·0	8·2	21·1
Eragrostiella leioptera Stapt, Bor.	—	12·7	40·2
Ischaemum goeblii Hack.	—	52·3	21·9
Osbeckia crinita Benth.	51·5	4·7	23·0
Annuals			
Borreria articularis (L.F) N F William.	—	—	29·5
Dimeria fuscescens Trin.	—	12·1	17·1
Eulalia quadrinervis (Hack) O Ktze.	10·0	—	—
Fimbristylis complanata Link.	—	38·5	26·6
Fimbristylis thomsonii Beeck.	20·1	18·0	2·9
Gentiana quadrifaria Clark non. Bl.	—	24·0	13·2
Osbeckia capitata Benth.	10·2	20·8	18·0
Paspalum arbiculare Forst.	10·5	1·8	10·9
Plectoranthus japonicus Burm (F.) Koidz.	16·3	3·1	—
Pogostemon quricularis (L.) Haosk.	3·1	10·7	2·4
Setaria glauca (L.) P Beauv.	14·0	5·6	—
Others*	80·0	56·9	53·3

*Species with IVI value less than 10.

Nutrient concentration of the soil down the 10 cm soil depth, in July is given in table 2. In general, the soil under *Osbeckia* type was richer in nutrients whereas that under *Eragrostiella* type was poorer. These differences were more pronounced for carbon, total nitrogen and available phosphorus than for NO_3-N and potassium.

All the growth parameters of *Arundinella*, *Carex* and *Chrysopogon* declined sharply ($P<0{\cdot}001$) in the nutrient poor soil under *Eragrostiella*–type (table 3). The decline in aboveground/belowground ratio, however, was significant at $P<0{\cdot}05$ level only. *Ischaemum* from frequently burnt site had lower value for this ratio and *Eragrostiella* had the least value. However, the values for *Ischaemum* and *Eragrostiella* are comparable to the values for *Arundinella*, *Carex* and *Chrysopogon* from nutrient poor sites.

Relative growth rates and net assimilation rates were generally higher for *Arundinella*, *Carex* and *Chrysopogon* in nutrient rich soil than in nutrient poor soil ($P<0{\cdot}001$), with maximum values for *Carex* (table 4). *Eragrostiella* in a desertified site had the least value. Leaf area ratio was very low for *Eragrostiella* followed by *Ischaemum*. The other 3 species in nutrient rich soil had generally higher leaf area ratio ($P<0{\cdot}05$) compared to nutrient poor soils, except for *Carex* where the reverse was true.

Concentration of nitrogen in *Arundinella* and *Chrysopogon* was higher in a nutrient poor soil than in a nutrient rich soil, whereas *Carex* showed a reverse trend ($P<0{\cdot}05$) (table 5). Inspite of low fertility status of the desertified site, concentration of nutrients in *Eragrostiella* was comparable to some of the species from nutrient

Table 2. Soil analysis (0–10 cm depth) at different sites at Cherrapunji in north-eastern India.

	Vegetation type		
Nutrient	*Osbeckia*	*Ischaemum*	*Eragrostiella*
Organic carbon (%)	2·0 ± 0·01	1·9 ± 0·05	1·4 ± 0·01
Total nitrogen (%)	0·29 ± 0·01	0·21 ± 0·001	0·15 ± 0·01
NO_3-N (mg/100 g)	0·12 ± 0·003	0·12 ± 0·001	0·10 ± 0·004
PO_4-P (mg/100 g)	0·29 ± 0·01	0·10 ± 0·001	0·08 ± 0·003
Potassium (mg/100 g)	0·12 ± 0·01	0·10 ± 0·004	0·10 ± 0·003

Table 3. Growth characteristics (± SE mean) of the different species at Cherrapunji in north-eastern India.

Species	Height (m)	Aboveground biomass (g)	Belowground biomass (g)	Aboveground/ belowground ratio
Arundinella	0·75 ± 0·005	77·96 ± 3·96	82·06 ± 4·17	0·95
	(0·50 ± 0·003)	(27·80 ± 1·18)	(32·71 ± 1·39)	(0·85)
Carex	1·10 ± 0·010	70·78 ± 3·79	67·41 ± 3·61	1·05
	(0·45 ± 0·032)	(14·04 ± 1·20)	(15·60 ± 1·33)	(0·90)
Chrysopogon	0·75 ± 0·061	73·74 ± 3·90	81·93 ± 4·33	0·90
	(0·30 ± 0·021)	(22·64 ± 1·71)	(30·19 ± 2·28)	(0·75)
Ischaemum	0·45 ± 0·035	46·60 ± 2·24	58·25 ± 2·80	0·80
Eragrostiella	0·35 ± 0·002	29·92 ± 1·99	46·03 ± 3·06	0·65

Numbers in parentheses indicate species grown in nutrient poor soil.

Table 4. Mean values (± SE of mean) of growth functions for the different species at Cherrapunji in north-eastern India.

	Growth functions		
Species	Relative growth rate (mg. mg^{-1} day^{-1})	Net assimilation rate (mg (cm^2)$^{-1}$ day^{-1})	Leaf area ratio (cm^2 mg^{-1})
Arundinella	0·0135 ± 0·009	0·123 ± 0·0114	0·117 ± 0·0084
	(0·0095 ± 0·0006)	(0·105 ± 0·0035)	(0·085 ± 0·0054)
Carex	0·0245 ± 0·0017	0·221 ± 0·0151	0·159 ± 0·0114
	(0·0147 ± 0·0011)	(0·135 ± 0·0115)	(0·251 ± 0·0181)
Chrysopogon	0·0106 ± 0·0008	0·121 ± 0·0115	0·082 ± 0·0075
	(0·0026 ± 0·0002)	(0·031 ± 0·0024)	(0·070 ± 0·0062)
Ischaemum	0·0116 ± 0·0012	0·125 ± 0·0101	0·013 ± 0·0011
Eragrostiella	0·0078 ± 0·0061	0·095 ± 0·0071	0·005 ± 0·0003

Numbers in parentheses indicate species grown in nutrient poor soil.

Table 5. Mean tissue concentration of nutrients (± SE mean) during the growth period of different species at Cherrapunji in north-eastern India.

	Nutrient concentrations (%)		
Species	Nitrogen	Phosphorus	Potassium
Arundinella	0·667 ± 0·045	0·098 ± 0·0075	0·733 ± 0·061
	(0·983 ± 0·075)	(0·099 ± 0·0071)	(0·630 ± 0·050)
Carex	0·925 ± 0·065	0·102 ± 0·0091	1·091 ± 0·091
	(0·801 ± 0·071)	(0·135 ± 0·0101)	(1·121 ± 0·101)
Chrysopogon	0·917 ± 0·081	0·168 ± 0·085	0·630 ± 0·051
	(1·283 ± 0·101)	(0·152 ± 0·089)	(0·82 ± 0·071)
Ischaemum	0·85 ± 0·071	0·114 ± 0·0095	0·59 ± 0·041
Eragrostiella	0·667 ± 0·051	0·106 ± 0·0080	0·53 ± 0·041

Numbers in parentheses indicate species grown in nutrient poor soil.

richer soil; the concentration of potassium in *Ischaemum* and *Eragrostiella* was, however, significantly lower than in others ($P > 0{\cdot}05$).

Starting with an initially high belowground biomass, a decline was observed in all the species in July–August, followed by an increase towards the latter part of the growing season (figure 1). Leaf biomass was initially much higher declining during the latter part. In *Arundinella, Carex* and *Chrysopogon*, belowground biomass was significantly higher ($P < 0{\cdot}05$) in the nutrient poor soil than in nutrient rich soil, with highest values recorded for *Eragrostiella* and *Ischaemum*. It may be noted that *Eragrostiella* and *Chrysopogon* have no stem component, as the leaves arise directly from the rhizomatous base. Further, seed production in *Chrysopogon*, did not occur in the nutrient poor soil.

Nitrogen allocation to belowground organs of *Arundinella, Carex* and *Chrysopogon* was lower ($P < 0{\cdot}01$) in nutrient poor soils than in the richer soils (figure 2). While in *Arundinella* the allocation to the stem was higher in the nutrient poor soil, ($P < 0{\cdot}05$) the reverse was true for *Carex*. Leaf allocation of nitrogen was significantly higher ($P < 0{\cdot}01$) for *Arundinella* and *Carex* but lower for *Chrysopogon* ($P < 0{\cdot}01$), in nutrient poor soils. Belowground allocation of nitrogen was maximum in *Eragrostiella* followed by *Ischaemum*.

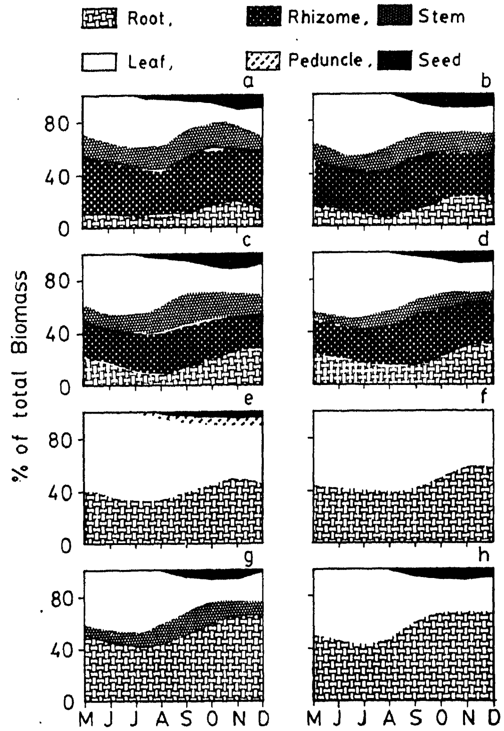

Figure 1. Growth and allocation strategies of *Arundinella* (**a**, **b**), *Carex* (**c**, **d**) and *Chrysopogon* (**e**, **f**) in grasslands at Cherrapunji in north-eastern India, under nutrient rich (**a**, **c**, **e**) and nutrient poor (**b**, **d**, **f**) sites and of *Ischaemum* (**g**) and *Eragrostiella* (**h**).

Allocation of phosphorus to the root component of *Arundinella*, *Carex* and *Chrysopogon* was significantly higher ($P < 0.05$) in nutrient poor than in nutrient rich soil (figure 3). Phosphorus allocation to the rhizome, however, was significantly higher on nutrient rich soil only in *Arundinella*. Allocation to the leaf component was significantly higher in nutrient poor soil ($P < 0.01$) in *Arundinella* and *Carex* but not very different in *Chrysopogon*. Maximum allocation of phosphorus to the root component was observed in *Ischaemum* and *Eragrostiella*. Allocation to the leaf and the seed components in *Eragrostiella* was higher ($P < 0.05$) than in *Ischaemum*.

Allocation of potassium to the belowground parts in *Arundinella* alone was significantly lower ($P < 0.05$) in nutrient poor than in richer soil (figure 4). Maximum allocation of potassium to the root component was observed in *Eragrostiella*. Allocation to the leaf component was maximum in *Ischaemum*.

A significantly lower ($P < 0.01$) nutrient uptake efficiency in nutrient poor than in richer soils was observed only for nitrogen (table 6), with least values for *Ischaemum*.

A significantly higher ($P < 0.05$) nitrogen use efficiency was seen for *Arundinella* and *Chrysopogon* in nutrient rich than in nutrient poor soil (table 7). *Eragrostiella* had maximum values for nitrogen and potassium use efficiency compared to all other species; even phosphorus use efficiency of this species was high. While *Chrysopogon* had a higher phosphorus use efficiency in nutrient poor than in rich soil, the reverse was true for *Carex*. Potassium use efficiency of *Chrysopogon* in nutrient poor soil was lower than in richer soil.

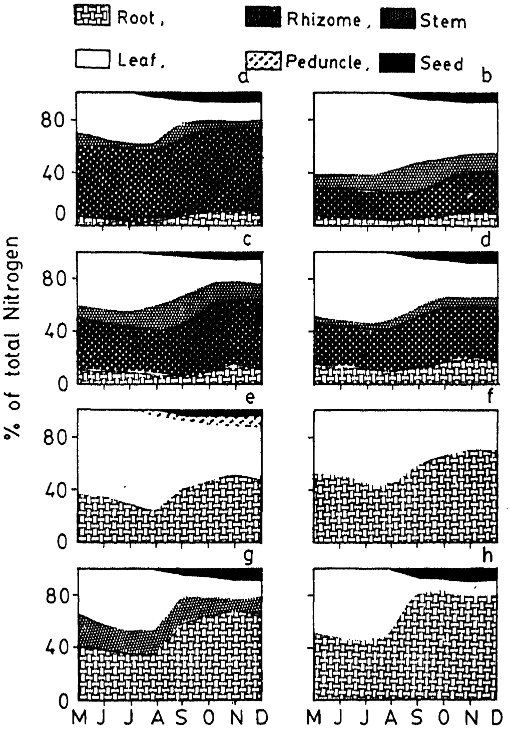

Figure 2. Same as figure 1; allocation of nitrogen.

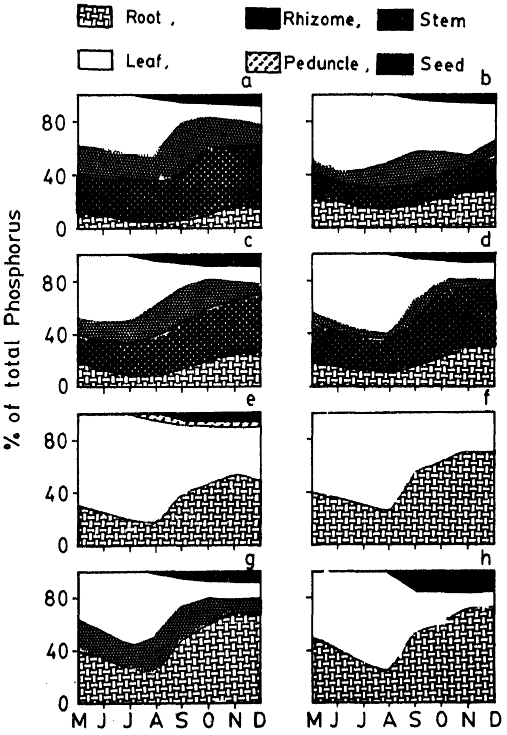

Figure 3. Same as figure 1; allocation of phosphorus.

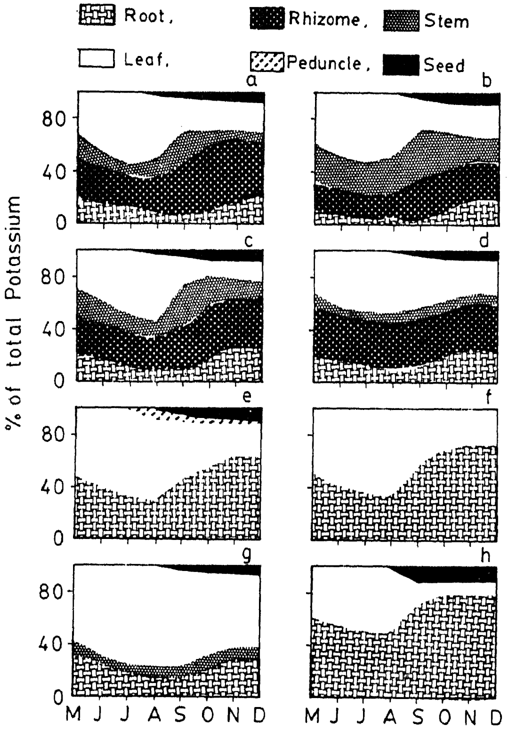

Figure 4. Same as figure 1; allocation of potassium.

Table 6. Nutrient uptake efficiency during annual growth period (mg nutrient absorbed/g root biomass) of different species at Cherrapunji in north-eastern India.

	Nutrient uptake efficiency		
Species	Nitrogen	Phosphorus	Potassium
Arundinella	107·02±8·5	11·7±9·5	53·05±5·1
	(56·05±4·2)	(10·4±8·9)	(58·13±4·9)
Carex	71·50±4·1	13·76±11·5	71·5±4·5
	(61·50±5·0)	(11·39±10·1)	(68·1±4·1)
Chrysopogon	102·10±8·9	12·83±1·9	54·09±5·0
	(88·09±7·5)	(11·51±2·0)	(53·66±5·1)
Ischaemum	45·69±4·3	12·37±0·10	47·05±0·6
Eragrostiella	65·13±6·1	13·40±1·5	60·67±4·10
Least significant difference ($P=0{\cdot}01$)	6·53	1·62	7·88

Numbers in parentheses indicate species grown in nutrient poor soil.

5. Discussion

The 3 grasslands under consideration here are degraded types with soils that are relatively deficient, compared to the climax forest type of the area (Khiewtam 1986). However, among these 3 grasslands, the soil under *Osbeckia*–type is relatively nutrient rich compared to that under *Eragrostiella*–type, the *Ischaemum*–type falling

Table 7. Nutrient use efficiency (mg dry matter produced/mg nutrient absorbed) of different species at Cherrapunji in north-eastern India.

	Nutrient use efficiency		
Species	Nitrogen	Phosphorus	Potassium
Arundinella	149·9 ± 12·5	1020·4 ± 95·4	136·4 ± 12·5
	(101·7 ± 9·7)	(1010·1 ± 91·7)	(158·7 ± 14·5)
Carex	108·1 ± 9·1	980·4 ± 75·9	91·7 ± 8·5
	(124·8 ± 11·1)	(740·4 ± 61·4)	(89·2 ± 7·1)
Chrysopogon	109·1 ± 9·4	595·2 ± 41·5	158·7 ± 13·7
	(77·94 ± 5·4)	(657·9 ± 51·3)	(122·0 ± 10·5)
Ischaemum	117·6 ± 9·7	877·2 ± 73·4	169·5 ± 14·3
Eragrostiella	150·9 ± 12·7	943·40 ± 81·4	188·7 ± 17·1
Least significant difference ($P = 0{\cdot}01$)	3·99	31·1	4·86

Numbers in parentheses indicate species grown in nutrient poor soil.

in between. It is therefore reasonable to expect larger biomass with a faster growth rate for the species under *Osbeckia*–type than under *Ischaemum* or *Eragrostiella* types. This is reflected not only through differences in the growth rate of species such as *Ischaemum* and *Eragrostiella* under these two grassland situations but also through differential growth rates of the other species on nutrient rich versus poor soils.

A useful strategy for survival of plant species growing in nutrient poor soils is to have greater allocation of biomass to the belowground organs as compared to aboveground parts. This is a mechanism to maximize nutrient intake through a larger root surface (Chapin 1980) rather than through a high nutrient absorption capacity (Nye 1977; Nye and Tinker 1977). This is seen in the present study too with respect to *Chrysopogon*, *Ischaemum* and *Eragrostiella*. *Eragrostiella* growing in a desertified site had maximum root biomass compared to all other species. This is also a mechanism to store organic food and nutrients in the belowground rhizomatous tissue and thus contribute to more efficient vegetative reproduction using the food reserves. Greater allocation of resources to belowground organs is thus an adaptation for survival under stress environment, S-strategy of Grime (1979). With larger allocation of available resources to the belowground organs of regeneration, species such as *Carex* and *Arundinella* have lesser allocation to sexual reproduction (Keeley and Keeley 1977). In fact, for the 3 tussock perennial grasses *Chrysopogon*, *Ischaemum* and *Eragrostiella* of stressed sites, with sprouts appearing from the base, the emphasis on sexual reproduction is minimal; this is unlike ruderal environment where resource availability is unpredictable.

An important characteristic that is of value for survival in a nutrient poor soil is a slow growth rate (Chapin 1980). Thus, species such as *Eragrostiella* had least growth rate compared to other species. Further, species occurring on and off nutrient rich soils such as *Arundinella*, *Carex* and *Chrysopogon* had drastically reduced relative growth rate and net assimilation rate in nutrient poor than richer soils. This helps to have comparable tissue concentrations of nutrients in nutrient poor soils as under nutrient richer soils. Thus, the reduction in nutrient concentration in the species growing under infertile soils is not very different from those growing in more fertile soils; in some cases the concentration is the same or even higher when under nutrient stress.

Species from infertile sites may have generally lower nutrient concentrations than from fertile sites, owing to ready availability and absorption. However, slow growing plant species from infertile sites may respond to nutrient stress by maintaining high tissue nutrient concentration through luxury consumption and or reduced growth rate (Auclair 1977). Contrary to this more rapid growth of a species in fertile sites may have dilution effect on the plant nutrient pool. Consequently, tissue concentrations of wild plants are less sensitive indicators of soil availability (van den Driessche 1974).

It is interesting to note that a C_3 species such as *C. cruciata* showed a higher growth rate compared with some C_4 species. This is perhaps related to a much higher leaf area ratio for this species compared with others. A similar observation was made by Saxena and Ramakrishnan (1984) while comparing C_3/C_4 strategies of early successional herbs after slash and burn agriculture (Saxena and Ramakrishnan 1981, 1983), with a C_3 species such as *Eupatorium odoratum* having a higher growth rate due to higher leaf area ratio. For comparing the productive potentials of C_3 and C_4 plants the importance of the extent of the light interception surface has been emphasized by a number of workers (Bull 1971; Caldwell 1974; Hafstra and Steinstra 1977) rather than the photosynthetic rate per unit leaf area as stressed by others (Black *et al* 1969; Black 1971). Gifford (1974) concluded that the large potential advantage of the C_4 species is progressively attenuated while moving from microscopic to macroscopic parameters and there remains no apparent difference between the two photosynthetic pathways at the level of crop growth rate when each species is grown in its own preferred environment.

The C_4 species, having a generally higher nutrient uptake and use efficiencies compared to C_3 species, are adapted to occupy nutrient deficient soils (Brown 1978; Saxena and Ramakrishnan 1983, 1984). It is therefore reasonable to find that *Ischaemum* and *Eragrostiella* types of grassland on relatively nutrient poorer soils have more C_4 species than the *Osbeckia* type under nutrient richer soils (Ram 1986).

The results, presented here, thus show a close adaptation of species to the environmental conditions in which they occur. Under nutrient stress conditions, as exists in many of these grassland types, the allocation strategy is largely geared to clonal propagation, with greater emphasis on C_4 species that are generally nutrient use efficient.

Acknowledgement

This work was supported by a research grant from the University Grants Commission, New Delhi.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 1, February 1988, pp. 25–30.
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Distribution of fungal spores within and above a crop of rice

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MS received 15 August 1986; revised 6 November 1987

Abstract. By sampling air with rotorods at different heights above ground level and at different times of the day it was possible to characterise the diurnally changing abundance of fungal spores and also the vertical profiles of changing concentrations within and above a crop of rice at Bhimavaram, Andhra Pradesh.

The spores of some fungi, notably *Nigrospora*, *Myrothecium* and *Cladosporium* were more abundant between 0800 and 1100 h than at other times of the day. In contrast maximal concentrations of *Leptosphaeria* spores were detected between 2400 and 0300 h. Idealised theoretical profiles showing logarithmic decreases in spore concentrations with increasing height were always obtained when considering total spora but for individual spore types they were only obtained during their periods of maximum atmospheric concentrations. Unlike the profiles of other fungi those of the false smut fungus *Ustilaginoidea virens* tended to show increasing concentrations both above and below crop level.

Keywords. Fungal spores; vertical distribution; turbulence; terminal velocity; airspora; rice crop.

1. Introduction

Although a large amount of information has been obtained on the changes in spore concentrations with height from 30 m above ground level, very little is known about vertical concentration profiles nearer to ground (Gregory 1973). Spore concentrations within and above these crops were recently measured (Gregory 1954; Last 1955; Schenck 1968; Eversmeyer *et al* 1973; Smith and Crosby 1973; Subba Reddi and Ramakrishna 1978; Subba Reddi *et al* 1978).

Rice is a major food crop in India. In most rice growing regions two crops are taken each year. A variety of parasitic and saprobic fungi flourish on rice (Mishra and Srivastava 1971; Padmanabhan 1974; Srivastava 1982). Preliminary experiments by Ramalingam (1962) on the distribution of these fungi within and above rice crops revealed steep concentration gradients. However, the concentration trends of some fungi differed from the average. To explore these variations, airspora samples were collected at Bhimavaram (16° 32′N and 81° 32′E) in Andhra Pradesh, where rice is widely grown in the kharif (June–November) and rabi (November/December–March) seasons.

2. Materials and methods

Perkins (1957) type rotorods were used for sampling the atmosphere. They are rotating-arm samplers recommended for sampling periods of up to 2 h (Edmonds and Benninghoff 1979); they catch particles ranging from 2·5–15·0 μm with reasonable efficiency (Batchelder 1977).

Air samples were obtained simultaneously at 0·15, 1·68, 3·20 and 4·72 m above ground level (see Atluri *et al* 1981). They were collected on 5/6, 18/19, 22/23 March during the 1983 rabi season. Thirteen samples, each of 1 h, were taken at intervals of 2 h on each occasion. The crop was 85 cm high and was at the seed-setting stage. By 5/6 March leaves were almost dry except for some of the upper ones, but by the time observations were made on 18/19 March all leaves were completely dry.

After being exposed, sticky cellotape strips on the leading edges of the upright rotorod arms were mounted on clean labelled microscope slides with glycerine jelly and then covered with long cover slips. They were then scanned and numbers of spores counted in fields 220 μm wide and 2 mm apart; numbers of spores per m^3 of air were calculated.

3. Results

To illustrate the changing atmospheric spore concentrations in vertical profiles through the crop of rice, a few spore types typical of either dry or wet spores were examined in detail. The dry spore types included *Nigrospora*, *Cladosporium*, *Myrothecium*, *Trichoconis*, *Ustilaginoidea virens* and *Periconia* whereas the wet spore element included the ascospores of *Leptosphaeria eustomoides*. Spores of these fungi occurred in concentrations sufficient to permit analysis.

By combining the data for the different sampling heights it was possible to discern from the geometric means 3 distinct types of diurnal variations (table 1). Concentrations of spores of *Nigrospora*, *Myrothecium* and *Cladosporium* were maximal between 0800–1100 h; those of *Ustilaginoidea* between 1000–1500 h and of *Leptosphaeria* between 2400 and 0300 h when atmospheres are predictably moist.

In addition to diurnal differences there were contrasting patterns of vertical distribution (figure 1). Spore concentrations within the crop tended to be greater, much greater in regard to *Leptosphaeria*, than those above the crop excepting *U. virens*, the false smut fungus of rice that colonizes spikelets. However, this generalisation is only really applicable during periods of maximal spore concentrations. At other times of the day when spore concentrations were small, they were highly variable. Those profiles of *Nigrospora* observed during 0900–1500 h, *Cercospora* between 0900–1300 h, *Myrothecium* between 0700–1500 h, *Trichoconis* between 0900–1100 h, *Periconia* between 0700–1300 h, *Cladosporium* from 0900 to 1500 h, *U. virens* from 1100–1500 h and *L. eustomoides* during the night (0100–0300) (table 1, figure 1) were smooth with concentrations above the crop progressively decreasing with increasing height.

4. Discussion

When spores are released into the atmosphere, they are distributed horizontally and vertically by atmospheric turbulence with the strength of horizontal diffusion usually being greater than that of vertical diffusion (Subba Reddi 1975). Therefore atmospheric spore suspensions are stirred by frictional turbulence and buoyancy which counteract downward sedimentation under gravity. In stable conditions spore concentrations theoretically decrease logarithmically with increasing height if the sources are near to ground level (Gregory 1967, 1973). This being so, there should be

Table 1. Diurnally changing concentrations (no. per m^3) of spores of different fungi at different heights within and above a crop of rice sampled on 5/6, 18/19 and 22/23 March 1983 (concentrations are geometric means).

	Time of day												
Spore type	1200–1300	1400–1500	1600–1700	1800–1900	2000–2100	2200–2300	2400–0100	0200–0300	0400–0500	0600–0700	0800–0900	1000–1100	1200–1300
A. Peak concentrations between 0800–1100 h													
Nigrospora	775	425	155	45	20	10	15	5	20	100	425	1200	525
Cercospora	65	60	10	5	5	0	0	0	5	40	375	250	135
Myrothecium	975	600	210	180	145	120	220	600	375	1400	2750	2125	1075
Trichoconis	30	30	5	0	0	0	0	0	0	15	90	90	40
Cladosporium	190	160	15	30	5	20	15	10	20	20	275	675	275
Periconia	30	25	10	15	10	0	5	0	15	80	70	110	60
B. Peak concentrations between 1000–1500 h													
U. virens	500	600	40	30	0	0	0	0	0	5	130	575	650
C. Peak concentrations between 2400–0300 h													
L. eustomoides	0	0	0	0	80	105	500	275	205	20	0	0	0
Total	3250	2150	600	350	375	350	825	1100	800	2100	7250	10500	5000

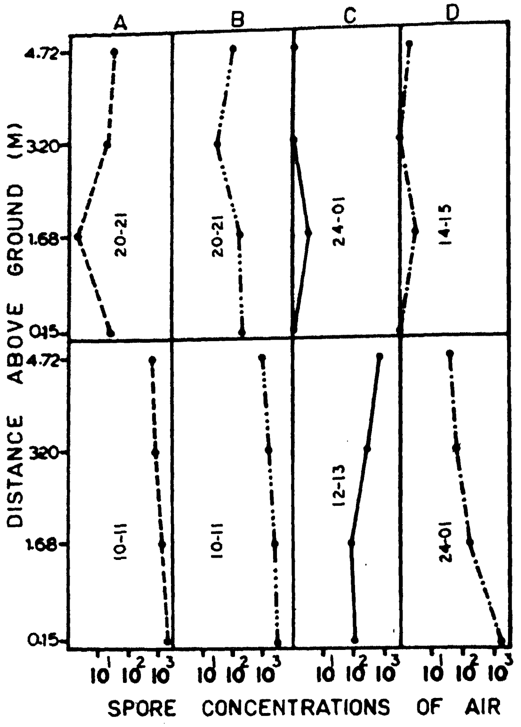

Figure 1. The patterns of vertical profiles in the hours of maximal spore concentrations (lower tier) and in the hours of minimal concentrations (upper tier).

a straight line relationship when logarithms of spore concentrations are plotted against logarithms of height. The present study revealed that this idealised relationship only occurred in the periods of rapid spore liberation, an observation in conformity with the findings of Atluri *et al* (1981), who measured spore concentration changes near the ground covered by natural vegetation.

The decrease in spore concentrations at different heights was not of the same order for different spore types even in a single test as revealed by the calculated ratios of spore concentrations. The different spore types may have different terminal velocities, and this factor might have resulted in the differences in the rate of spore concentration decrease. The ratios of spore concentrations for the same spore type at the successive heights also were not the same indicating that the change in spore concentration levels from height to height was not of the same magnitude, which property obviously be related to the vertical air temperature gradient. Last (1955), Eversmeyer *et al* (1973) and Atluri *et al* (1981) reported similar trends in the changes of spore concentrations.

Last (1955) observed that the changes in spore concentrations within and above the crop are affected by the conditions at the site where the spores are formed and liberated. He found that *Ustilago* spores are liberated from the ears and were almost as abundant at the top as in the lower part of the canopy, while *Cladosporium* and *Erysiphe* spores are produced on the foliage lower in the crop and were more than 3 times as concentrated at the base as at the top of the crop. The pattern of changes in spore concentrations of *U. virens* produced in the top canopy layer of the rice crop resembled that of *Ustilago*. Those of the spore types produced lower in the crop were alike those of *Cladosporium* and *Erysiphe*.

The magnitude of air turbulence is a function of vertical temperature gradient which varies with the time of day, and consequently the turbulence factor varies in intensity during the day. Low turbulence conditions normally exist during night. During daylight hours superadiabatic lapse conditions develop at sunrise and turbulence is highest at noon in India. Accordingly, spore concentrations vary with the time of day and the rate of decrease with height is less rapid at night. This tendency was evident in the present study. Ascospores of *L. eustomoides* are usually liberated at night in association with high humidities and were recorded in quantity during 0100–0300 h. The decrease in ascospore concentrations of this nocturnally spread fungus was rather more rapid than that of the day liberated spore types. These observations are in line with those of Atluri *et al* (1981).

In contrast to the trends displayed by individual spore types, those of total fungal spore flora always inclined to a decreasing trend in concentrations with increasing height, because the factors governing spore formation and release put vastly differing numbers of spores of different fungi into the atmosphere at different times of the day.

5. Conclusion

Although spore concentrations decreased with increasing height from ground level, the decrease was not steep. Also the concentrations within the crop were not much larger than those above the crop. Perhaps the vast acreage of rice fields forming a large area source supporting abundant fungal biomass and the short interval between sampling heights might be the factors that did not allow a steep decrease in spore concentrations. It is thus suggestive that over large area source trapping the airspora well above the source reflects the changes in spore concentrations within the crop.

Acknowledgements

The authors are grateful to Prof. R L Edmonds, College of Forest Resources, University of Washington, USA, Prof. F T Last, Forestry and Natural Resources, Edinburgh University, Scotland, UK for helpful suggestions, and the Principal and management of DNR College, Bhimavaram for the facilities.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 1, February 1988, pp. 31–39.
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Morphological aspects and taxonomical prospects of leaf idioblasts in Pandaceae and Caryocaraceae

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MS received 24 April 1987

Abstract. Sclereids and tracheoids are found to be key characters to distinguish the taxa included under the Pandaceae. Their presence or absence appears to be a strong evidence that *Microdesmis* and *Centroplacus*, and *Panda* and *Galearia* can be grouped under two sections, anatomically. The vein termini sclereids in *Anthodiscus* and brachytracheoids in *Caryocara* of the Caryocaraceae are useful to distinguish the two genera from one another.

Keywords. Foliar idioblasts; sclereids; tracheoids; pandaceae; caryocaraceae; taxonomic prospects.

1. Introduction

A perusal of plant anatomical literature reveales that out of the 4 genera of Pandaceae viz. *Centroplacus* Pierre, *Microdesmis* Hook., *Galearia* Zool and Mor., and *Panda* Pierre, varied idioblasts, especially sclereids are recorded only in *Centroplacus* and *Microdesmis*. They are also recorded in *Anthodiscus* G F W May, *Caryocar* Allam. ex L. of the Caryocaraceae (Metcalfe and Chalk 1950; Rao and Das 1979; Rao and Bhattacharya 1978). An investigation of the above taxa has been undertaken to examine the typology and distribution of leaf sclereids and tracheoids, with a view to record their potential systematic implications not so far made apparent by others (Silva 1968, 1969).

2. Materials and methods

Leaves of herbarium specimens were restored using Aerosol OT at 45°C for 3 days and washed in water before subjecting to clearing and staining by basic Fuchsin and KOH treatment as suggested by Page and Tan (1986) to produce a deeply stained 3-dimensional vascular skeletons of the leaf *in-situ* within a transparent whole leaf body. This technique is found to be helpful to study vein ramifications and their elements in greater details.

3. Pandaceae

3.1 *Materials examined*

The following herbarium leaf specimens belonging to 11 species of 4 genera were examined: *Panda oleosa* L. Africa, Gabon, Nkoulounga, G Touzet 154 (P); *Galearia aristifera* Miq., Borneo, Sulaiman 5 (L); *G. celebica* Koord, New Guinea, Schodde and Craven 4240 (L); *G. fulva* (Tul.) Miq., Malaya, *s.l.* FRI 2850 (L); *Microdesmis* *yafungana* Leonard, Africa, Congo, Gilbert 2155 (L); *M. pierlotiana* Leonard, Africa, Boukoko, Tiroerand 1175 (L); *M. caseariifolia* Planch. Thailand, Geesink and Santsuk 5185 (L); *M. puberula* J D Hook. f. ex Planch. Cameroun, Leeuwenberg 5935 (P); Congo, Leonard 467 (L); *M. magallanensis* (Elm.) Steen. Philippines, Sibuyan, Elmer 12323 (L); *M. zenkeri* Pax. Africa, Gabon, de Libreville, R P Klaine 2731 (L); *Centroplacus glaucinus* Pierre, Gabon, Abanga, N Halle 2403 (P).

3.2 *Observations*

3.2a *Panda* is a tropical west African monotypic genus which is represented by a single species, *P. oleosa*.

The vein reticulum and the veinlets in the leaves are accompanied with a few slender thick-walled fibres. The borders of the areoles are sheathed with thin-walled parenchymatous cells which are rarely observed to accompany the veinlets. However, the vein endings along with brachytracheoids possess thin-walled more or less enlarged cells of parenchymatous nature (figure 1). Unlike these unspecified cells, the brachytracheoids possess sparsely to densely pitted cell walls.

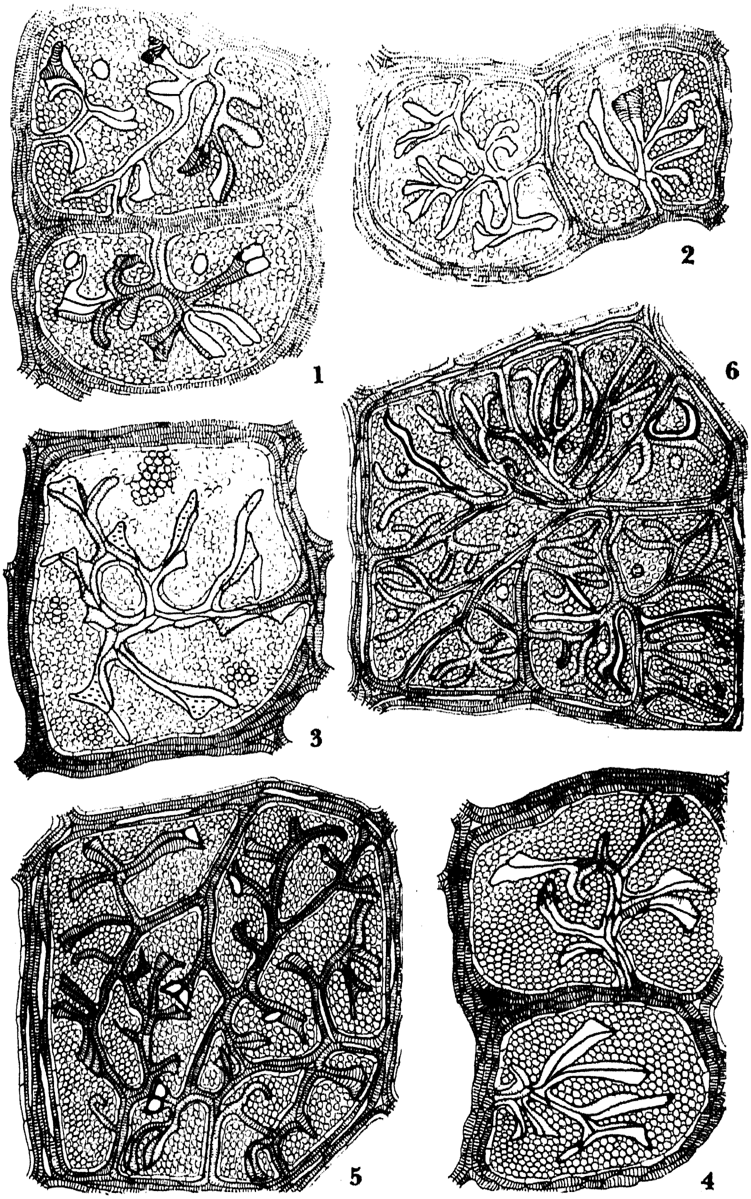
3.2b *Galearia*, a genus in a confused state taxonomically is represented by a number of variable species spread all over the south east Asia and Malaysia to Soloman Islands.

The vein reticula and the veinlets of the 3 species, viz *G. aristifera*, *G. celebica* and *G. fulva* are encased partially by elongated parenchymatous cells up to the apex of the veinlets. They are relatively thin-walled and more or less irregular in shape and size. However, at the vein ends there are thick-walled, irregularly shaped, pitted or non-pitted sclerotracheoids closely juxtaposed along with brachytracheoids. A striking trend of these terminal or sub-terminal cells observed is their close disposition with limited extension within the areole (figures 2–4).

3.2c *Microdesmis* is a tropical genus represented by 10 species spread over Africa, south east Asia and west Malaysia.

In all the investigated species of *Microdesmis* the vein reticula and the veinlets are bordered with thin-walled parenchymatous cells. The veinlets and the vein ends show distinct categories of idioblasts chiefly in the form of tracheoids and sclereids of varied shapes and sizes. In *M. yafungana*, *M. pierlotiana* and *M. caseariifolia* terminal sclereids and tracheoids are more or less tube-like and conspicuous (figures 6–8). In *M. puberula*, the terminal idioblasts are very much extended irregularly or sometimes they extend, especially the sclereids from one side of the reticulum to the opposite side following a serpentine course of orientation (figures 9, 11). They are strongly birefringent and their wall reaction is positive to phloroglucinol-HCl test. The tracheoids in this species are more or less strap shaped of varied length, uneven width and helically thickened with rounded ends. In the mesophyll profile they are observed to occupy and extend to different tissues. They are weakly birefringent under polarised light. They are comparable to that of loratotracheoids as observed in Penaeaceae (Rao and Das 1976, 1979; Rao 1987).

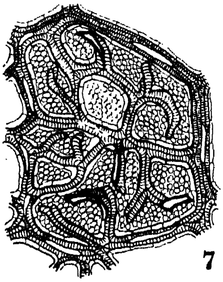
In *M. magallanensis*, however, there are distinct structural differences in veinlet syndrome when compared to the other species of *Microdesmis*. The vein endings possess sclerotracheoids or brachytracheoids in twos or threesome and short tube-like sclereids in close proximity (figures 5, 12). Apart from this, the other structural



Figures 1–6. Surface view (×250). **1.** *P. oleosa.* **2.** *G. fulva.* **3.** *G. celebica.* **4.** *G. aristifera.* **5.** *M. magallanensis.* **6.** *M. pierlotiana.*

differences which distinguish the other species of *Microdesmis* are the diamond or cube-shaped crystals only along the midrib profile, columnar spongy cells with plenty of air space and small sized thick-walled abaxial epidermal cells with depressed stomata (figure 12).

3.2d *Centroplacus* is a west African taxon represented by a single species, viz *C. glaucinus.*



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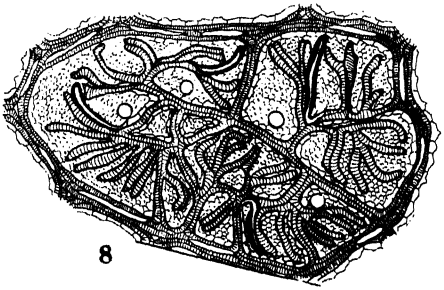

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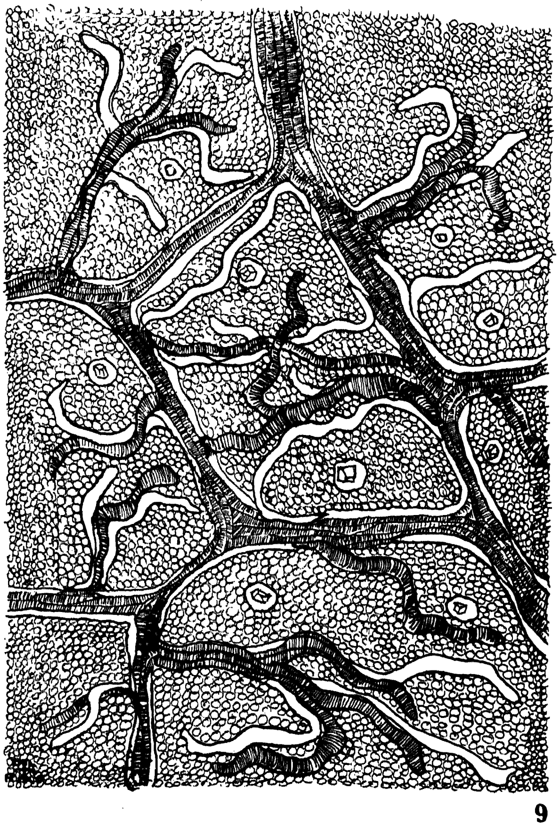

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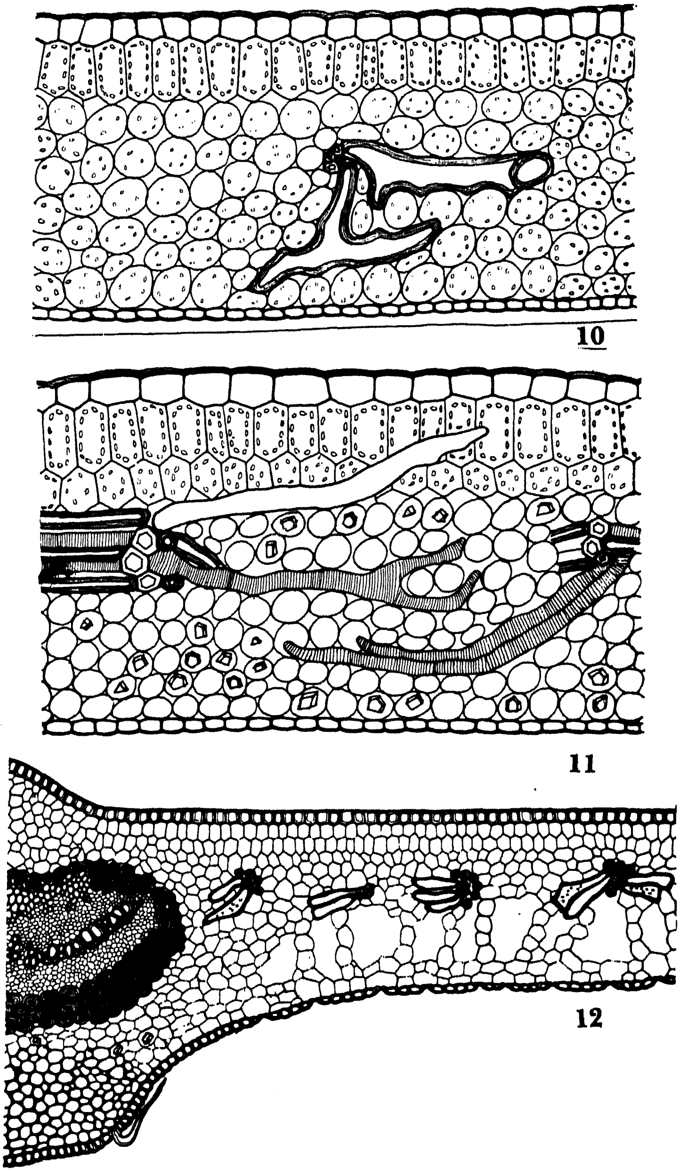
Figures 7–9. Surface view (× 240). **7.** *M. casearifolia.* **8.** *M. yafungana.* **9.** *M. puberula.*

The vein reticula and the veinlets are encased partially with irregularly shaped parenchymatous cells. The vein ends are characterised by sclereid-like cells. They vary from simple to oblong or tubular branched forms with rounded ends in close associations with the vein endings or closely accompanying the veinlet before protruding into adjacent mesophyll areas (figure 10). They extend often touching the vein reticula around the areoles. The varied forms have in them a sequence of intermediate forms. Notwithstanding their variations, they have relatively thin cell wall and large lumen. The outer cell wall is feebly undulating, weakly birefringent and positive to phloroglucinol-HCl test.



Figures 10–12. Transections (× 250). **10.** *C. glaucinus.* **11.** *M. puberula* **12.** *M. magallanensis.*

4. Caryocaraceae

A family of 2 genera *Anthodiscus* and *Caryocar* and about 25 species distributed mostly in tropical America and out of these only 4 species of *Anthodiscus* and 3 species of *Caryocar* have been examined.

4.1 *Materials examined*

Anthodiscus amazonica Gleenon, Brasil; Amazon, G T Prance *et al* 16435 (GH); *A. majorensis* Gilly, Surinam, Tafelbung, B Maguir 24576 (A); *A. montanus* Gleenon, Colombia, A E Lawrence 802 (A); *A. obovatus* Benth. Brasil, Rionigro, R Spruce 3146 (CAL); Colombia, R E Schulter 14660 (GH); *A. peruamus* Baill. Columbia, R E Schulter and F Topez 9387 (GH).

4.2 *Observations*

4.2a *Anthodiscus* is a south American tropical genus represented by 10 species.

The vein reticulum and the veinlets are encased by a few thick-walled sclerenchymatous fibres. The borders of the areoles possess irregularly shaped parenchymatous cells which are also found juxtaposed up to the veinlets apex. The ultimate vein endings in all the investigated species possess ovoid to sub-spheroidal sclereids irregularly pitted with moderate cell wall and wide lumen. This terminal disposition of sclereids appears to be a salient feature in *A. amazonica*, *A. majorensis*, *A. obovatus* (figures 13, 15) and *A. peruamus*. In *A. montanus*, however, they are restricted to vein endings occurring near the mid-vein portion of the leaf.

4.2b *Caryocar* is a genus of tropical America represented by 20 species (Pilger 1925).

4.3 *Materials examined*

Caryocar glabrum (Aubl.) Pers., R Spruce *s.n.* (CAL); Peru, Toreto, T Williams 4425 (GH); *C. microcarpum* Ducke, Brasil, Amazon, Prance *et al* 9112 (GH); *C. pallidium* A C Sm., Brasil, Amazon, Manaus, J E Paula 13 (INPA). *C. Villosum* (Aubl.) Pers. Brazil, Acre, Prance *et al* 2490 (GH).

4.4 *Observations*

The vein reticula and the veinlets in all the investigated species are accompanied by elongated sclerenchymatous fibres to a great extent. The veinlets have a few elongated fibres adjoining the veins. The vein endings possess short tubular ovoid brachytracheoids either solitary or in twos or threesome (figure 14). Further, it is not uncommon to observe a few disjointed brachytracheoids in the vicinity of veins. The brachytracheoids are thin-walled, weakly or strongly sclerefied and apparently appears to be of diagnostic feature of this genus.

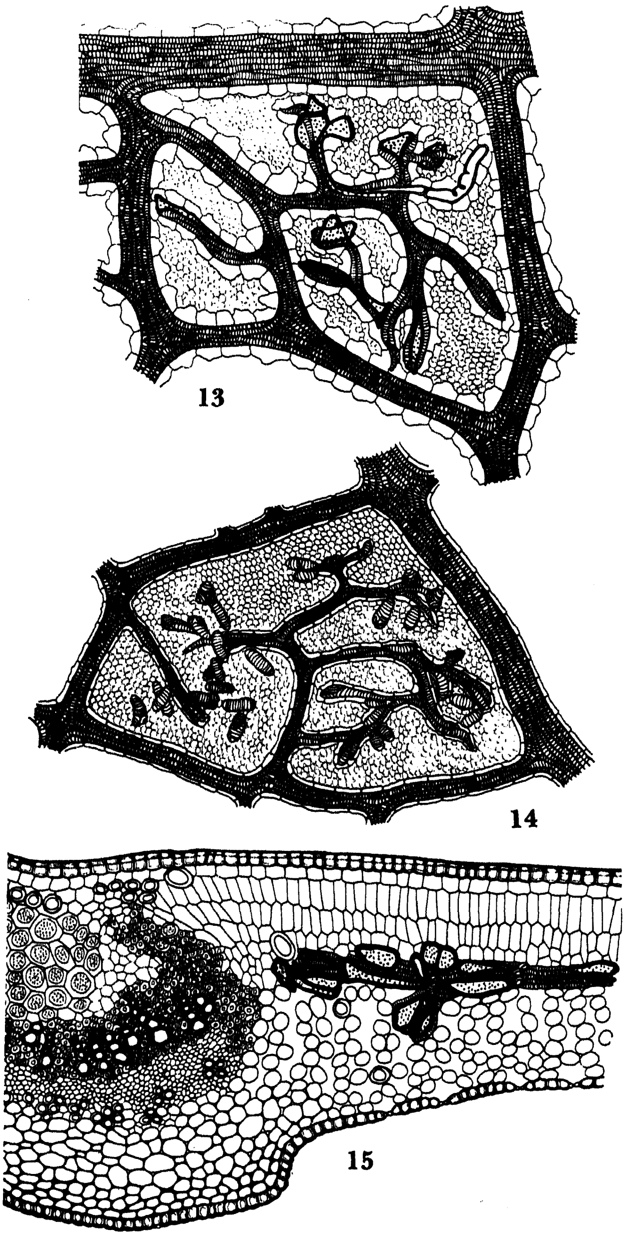
Another noteworthy feature in the mesophyll is the absence of sclereids of any category. However, the reported occurrence of sclereids in the vicinity of veins, and also along the leaf margins of *C. nuciferum* (Blank 1939) can be of exceptional diagnostic interest for this species.

5. Conclusion

It is clear that the morphological features of the vein systems of the Pandaceae especially the varied idioblasts form an additional feature of diagnostic interest at generic level. Based on their typological features the following tentative key is prepared as an aid in the identification of the genera of this family.

Vein terminal:

1. Sclereids present
    2. Veinlets with sclereids and tracheoids . . . . *Microdesmis.*
    2. Veinlets with sclereids . . . . *Centroplacus.*



Figures 13–15. 13 and 14. Surface view (×250). **13.** *A. obovata.* **14.** *C. glabrum.* **15.** Transection of *A. obovata* (×250).

1. Sclereids absent
    2. Veinlets with brachytracheoids .... *Panda.*
    2. Veinlets with sclerotracheoids .... *Galearia.*

The present study revealed that idioblasts, chiefly sclereids and tracheoids are the key characters to distinguish the 4 taxa included under this family. Their presence or absence appears to be a strong evidence that *Microdesmis* and *Centroplacus*, and *Panda* and *Galearia* can be separated under two sections, anatomically.

Microdesmis in general stands distinctly apart from *Galearia* and *Panda*. However, *M. magallanensis* shares vein ends similar to that of *Galearia* and *Panda*, and serves as a link with those genera. In this context the structural differences of *M. magallanensis* is all the more of taxonomic interest in view of its reduction from *Worcesteranthus* Merr. of the Olacaceae (Merrill 1914) to *Microdesmis* of the Pandaceae (Steenis 1955).

The vein termini idioblasts and their typological features in *Anthodiscus* and *Caryocar* of the Caryocaraceae are sufficient to distinguish the two genera from one another. The occurrence of terminal sclereids in *Anthodiscus* and terminal brachytracheoids in *Caryocar* are more or less distinct and can be utilised for generic circumscription and sub-division despite their shared anatomical features in respect of hairy covering, idioblasts and sub-epidermal origin of cork. Further, it is observed that these idioblasts conform to sclereids or tracheoids, and at no instance any intermediary hybrid forms as reported in a few species of *Memecylon* (Rao 1957), *Lijindinia* (Rao *et al* 1983) and *Mouriri* (Foster 1946) of the Melastomataceae were observed in any of the species examined. However, these tracheoid idioblasts are comparable with those described in *Reaumuria* of the Tamaricaceae (Rao and Chakraborti 1982), *Capparis* of the Capparidaceae (Rao and Das 1978, 1979, 1981), *Fouquieria* of the Fouquieriaceae (Lersten and Carvey 1974), *Boronia* of the Rutaceae (Rao and Bhattacharya 1978, 1981), *Euphorbia* of the Euphorbiaceae (Sehgal and Paliwal 1974), a few other species of the Scrophulariaceae (Verghese 1969) and the Cunoniaceae (Rao and Dickison 1985a, b).

Notwithstanding the minor tracheoidal differences at the vein termini of the investigated species of *Caryocar*, it is certain that they cannot be used to distinguish them at specific levels. It is clear, however that they form a part of syndrome of characters at generic level for any thorough taxonomic analysis. On the other hand, in *Anthodiscus*, the occurrence of sclereids relates well to the alliance of this taxon to the Theaceae in which, however almost all the taxa possess diffuse pattern of leaf sclereids. The orderly terminal pattern of sclereid idioblasts in *Anthodiscus* not only justifies its diagnostic value but also its separate identity as it has been possible to build a key at generic level.

Acknowledgements

The authors are thankful to Dr R A Howard of Harward University, USA, Dr N Hall'e of Paris Museum, Dr P Baas of Rijksherbarium, Leiden for the gift of leaf specimens, B V Shetty of the Botanical Survey of India, Calcutta and Miss Mary Gregory, Jodrell Laboratory of the Royal Botanic Gardens, UK for providing well related literature. One of the authors (TAR) is thankful to the University Grants Commission, New Delhi for the financial support.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 1, February 1988, pp. 41–48.
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New nodulating papilionoid legumes of potential value for revegetation of degraded ecosystems

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MS received 27 April 1987; revised 17 December 1987

Abstract. The north-western Himalayan ranges were surveyed for nodulating legumes that could be useful in tropical afforestation, soil amelioration and reclamation of open cast mine sites, mine spoil heaps, eroded slopes and other denuded ecosystems. Seven new nodulating papilionoid legumes were found and all of them have potential application in revegetation programmes. One new record of a non-nodulating papilionoid member is also reported. The ecological aspects of sampling sites, and habit and root characteristics of the legumes are described. The patterns of nodulation and morphology of nodules are also given. There is a substantial amount of polymorphism in nodule size and shape within a single species as well as among the species; a part of this variation is habitat induced. The nodulation occurs essentially during monsoon season. The remarkable similarity in growth form and nodulation patterns among 7 taxa is the consequence of adaptation to similar ecological conditions.

Keywords. Ecosystems; legumes; nodulation; north-western Himalaya.

1. Introduction

The Himalaya, the highest mountain range in the world, play a vital role in the continental distribution of climate. The wide latitudinal spread and the steep altitudinal gradient (from sea level to over 8000 m) permit a wide range of thermal conditions and insolation levels with local and regional variations in rainfall pattern. Consequently, many natural ecosystems exist within the Himalaya—from tropical rain-forest to alpine tundras, and from swamps to cold deserts. These ecosystems harbour many life forms, only a minor fraction of which is known to science. Because of increasing habitat pressures, some of the forest ecosystems are almost extinct (e.g. the frontline oak forest), and others are either converted into scrub or degraded into barren slopes.

One of the approaches for the restoration of ecological equilibrium in derelict Himalayan ecosystems is revegetation with plant species that are early colonizers and that enrich the soil. The legume—*Rhizobium* symbiosis has been used in the reclamation of marginal lands, eroded slopes, open cast mine sites, mine spoil heaps; agroforestry and tropical afforestation programmes (Dancer *et al* 1977; Haystead and Marriott 1978; Jeffries *et al* 1981a, b; Babu *et al* 1985).

There are about 19,000 species of legumes in the biosphere, of which only one-sixth have been examined for nodulation (Allen and Allen 1981). In Himalaya, the legumes are represented by 400 species belonging to 100 genera. Data on the nodulation of these legumes are almost lacking. In order to identify useful legume—*Rhizobium* associations for reclamation of denuded Himalayan ecosystems, field surveys were made for nodulating legumes. The present communication reports 7 nodulating papilionoid legumes and one record of a non-nodulating legume from north-western Himalayan ecosystems. The nodulative status of these 8 legumes were hitherto unknown (Allen and Allen 1981).

2. Materials and methods

Field trips were conducted during April–May (1984 and 1985) and July–September (1983, 1984 and 1985). Data on the habit and root characteristics were recorded. The spread of lateral roots and vertical penetration of main taproot were traced to the extremity, after removing the soil and or gravel carefully from the root zone. The phenology of each species was also recorded. The seedlings and plants at vegetative, flowering and or fruiting stages were examined for nodule occurrence. The seedlings close to mother plants were selected so as to ensure genetic identity of the plants examined. Plants (10 to 30) of each species were scored for the presence or absence of nodules. The pattern of nodulation, the morphology and colour of nodules, and the presence or absence of leghaemoglobin (based on visual observation of split-opened nodules) were recorded. For the isolation of rhizobia and for the structural examination, the nodules were carefully excised from the roots, after thorough washing in running water, dried superficially with bibulous paper and stored in vials containing glass wool placed over fused $CaCl_2$.

The soil temperature was measured near the rhizosphere at a depth of 6 cm by a telethermometer. The soil moisture content was determined by moisture-meter. The measurements of soil temperature and moisture were taken during April–May and July–September. The pH of the soil suspension (1 part of soil: 2 parts of distilled water) was determined with the help of a digital pH meter.

3. Results and discussion

Table 1 gives the regions, sampling sites, altitude and ecological aspects of the area surveyed. The information on the distribution, range, habit and root characteristics is given in table 2. All the species are perennials and are deep-rooted (the main tap root penetrated vertically to a depth of more than 60 cm) with a few lateral roots suggesting the stony or gravelly nature of the substratum except for *Caragana brevispina* and *Indigofera heterantha* which inhabit slopes with relatively thick soil layer. All the 8 species show restricted geographical distribution and are either confined to Himalaya of India and or extended to the neighbouring ranges of Pakistan, Afghanistan and China.

Table 3 gives the nodulative status, nodulation pattern, nodule morphology, and the presence or absence of leghaemoglobin of 8 legumes. Nodules were present on all the legumes, except *Chesneya cuneata.* The non-nodulation, in *Chesneya* is perhaps associated with reddish brown to red colour of roots which is due to the presence of a secondary metabolite. A similar correlation between nodulative status and colour of the roots has been reported in other taxa (Corby 1971). Except *I. heterantha*, the number of nodules per plant varies from 6–20 in all the species. In all the taxa the nodulation is mostly confined to secondary roots and or rootlets at a depth of 3–20 cm. The relatively high number of nodules per plant (more than 50) in *I. hete-*

Table 1. Field data of sampling sites.

Region	Location	Sampling site	Altitude (m)	Soil characteristics				Ecological aspects
				Texture	Temperature (°C)	Moisture (%)	pH	
Ladakh	34°6′ N 77°36′ E	Leh	3500	Sandy to gravelly	28–32	2–3	7·5–8·0	Cold desert; barren plateau with scattered individuals of *Chesneya* and *Echinops*
Valley of Flowers	30°48′ N 79°43′ E	Valley of Flowers	3355	Sandy-loam	11–14	10–14	5·2–5·6	Alpine zone; exposed to glacial migration. Moist alpine scrub with rhododendrons and alpine herbs such as primulas, saxifragas, gentianas and other caryophyllaceous members
		Ghangharia	3100	Clay rich sandy	12–15	20–24	5·2–5·6	Subalpine zone; subalpine forest with *Betula utilis*, *Abies pindrow* and *Taxus baccata*
Mussoorie range	30°27′ N 78°15′ E	Barlowganj	1700	Sandy to gravelly	26–30	20–25	7·5–7·9	Temperate zone; frontline oak forest with *Aesculus indica*, *Rhododendron arboreum* and conifers such as *Cedrus deodara* and *Cupressus*
		Bhatta	1500	Sandy to gravelly	28–32	1–7	7·7–8·0	Moist temperate mixed forest reduced to scrub due to grazing and cultivation; *Berberis*, *Lantana* and *Eupatorium* are common
		Chunakhala Limestone Mines	1400	Gravelly or stony	29–32	1–4·5	7·6–7·7	In the sheltered valleys vegetation is of subtropical moist mixed forest with *Bauhinia retusa* and stunted *Quercus* trees. On the slopes, forest is degraded to scrub of *Berberis* and *Eupatorium*

Table 2. Habit, root characteristics, uses and ecological significance of eight legumes.

Legume	Distribution range	Site of observation	Habit and root characteristics	Potential application
Argyrolobium flaccidum (Royle) Jaub. and Spach	NW India (plains to 1500 m)	Bhatta	Erect, diffuse, much-branched perennial herb. Deep rooted with 10–15 lateral roots and indeterminate primary root	Pioneer species
Astragalus graveolens Buch. -Ham. ex Benth.	NW Himalaya extending to Afghanistan and Pakistan (1500–4000 m)	Bhatta and Barlowganj	Erect perennial herb. Deep rooted with 5–10 lateral roots and indeterminate primary root; coppices well	Pioneer species; an excellent species for revegetation of mined areas
Astragalus leucocephalus Grah. ex Benth.	NW and Central Himalaya extending to Afghanistan (1500–4700 m)	Bhatta and Barlowganj	Perennial tufted herb. Deep rooted with 5–10 laterals and indeterminate primary root	Pioneer species
Caragana brevispina Royle	Kashmir to central Nepal (2400–3200 m)	Ghangharia	Erect, woody spinescent shrub. Deep rooted with more than 100 lateral roots and indeterminate growth of primary root	Windbreak hedge plant; young pods as vegetable; firewood crop, bark for fibre. Cold- and drought-resistant
Chesneya cuneata (Benth.) Ali	Kashmir, extending to Pakistan (2400–4000 m)	Leh	Perennial tufted herb with a thick woody tap root and rosette of leaves. Deep rooted with 5 or more laterals and indeterminate growth of primary root; the roots are brownish red to red	Pioneer species; extremely drought-resistant
Indigofera heterantha Wall. ex Brand.	Himalaya, extending to China (1300–3000 m)	Chunakhala Limestone Mines	Shrub to medium sized tree. Deep rooted with more than 100 laterals and indeterminate primary root; coppices well	An excellent fodder- and firewood-yielding tree. Early colonizer, excellent species for reforestation programmes
Lespedeza stenocarpa Maxim.	Himalaya (600–2000 m)	Bhatta	Woody shrub; deep rooted with 5–10 laterals and indeterminate primary root; coppices well	An excellent fodder- and firewood-yielding shrub. Early colonizer, excellent species for reclamation of mined areas
Thermopsis barbata Royle	Kashmir to SW China (3000–4600 m)	Valley of Flowers	Perennial herb with tufted stems. Deep rooted with 5–10 lateral roots and indeterminate growth of primary root	Pioneer species

Table 3. Nodule occurrence, nodulation patterns and morphology of nodules in 8 legume species.

Legume	Nodulation	Type of plants observed	Nodulation pattern	Nodule size[a] shape and colour	Leghaemo-globin
Argyrolobium flaccidum	N	S; V; F; Fr	6–20 nodules; mostly on secondary roots/rootlets, a few on the primary root; some in the axils of secondary roots, at 2·5 cm depth or more	Very small to large; elongate, bifurcate and or coralloid; cream outside and pink inside	+
Astragalus graveolens	N	S; V; F; Fr	6–20 nodules; on secondary roots/rootlets, at 4 cm depth or more	Medium; mostly globose, few elongate; pink outside as well as inside	+
Astragalus leucocephalus	N	S; V; F; Fr	1–5 nodules; mostly on secondary roots/rootlets; few on primary root, at a depth of 6 cm or more	Very small to medium; elongate, a few are bifurcate; brown outside and pink inside	+
Caragana brevispina	N	F; Fr	6–20 nodules; on secondary roots/rootlets at 20 cm depth or more	Very large; fan shaped and palmately 4–6 lobed; red outside and pink inside	+
Chesneya cuneata	Non	S; V; F; Fr	—	—	—
Indigofera heterantha	N	S; V; F; Fr	> 50 nodules on secondary roots/rootlets at a depth of 5 cm or more	Very small to medium; globose entire and globose bifurcate; brown outside, pink inside	+
Lespedeza stenocarpa	N	S; V; F; Fr	6–20 nodules; on secondary roots/rootlets at a depth of 10 cm or more	Very small to medium; globose; brown outside, pink or red inside	+
Thermopsis barbata	N	S; V; F; Fr	6–20 nodules; mostly on secondary root/rootlets; very few on primary root at a depth of 5 cm or more	Large; unbranched, perennial globose-apical; upper part of nodule creamish outside as well as inside; basal part yellow or red outside, pink inside	+

N, Nodulating; Non, non-nodulating; S, seedling; V, vegetative stage; F, flowering stage; Fr, fruiting stage; +, present.

[a] Nodule classification (based on size)

Very small: <2 × 1 mm

Small: upto 3 × 1·5 mm

Medium: upto 6 × 5 mm

Large: upto 15 × 7 mm

Very large: upto 17 × 20 mm.

rantha as compared to other legumes, can be explained on the basis that this species grows on wooded slopes where the soil strata were thick. The presence of copious leghaemoglobin in nodules of all the legumes suggests that nodules are effective and fix atmospheric nitrogen.

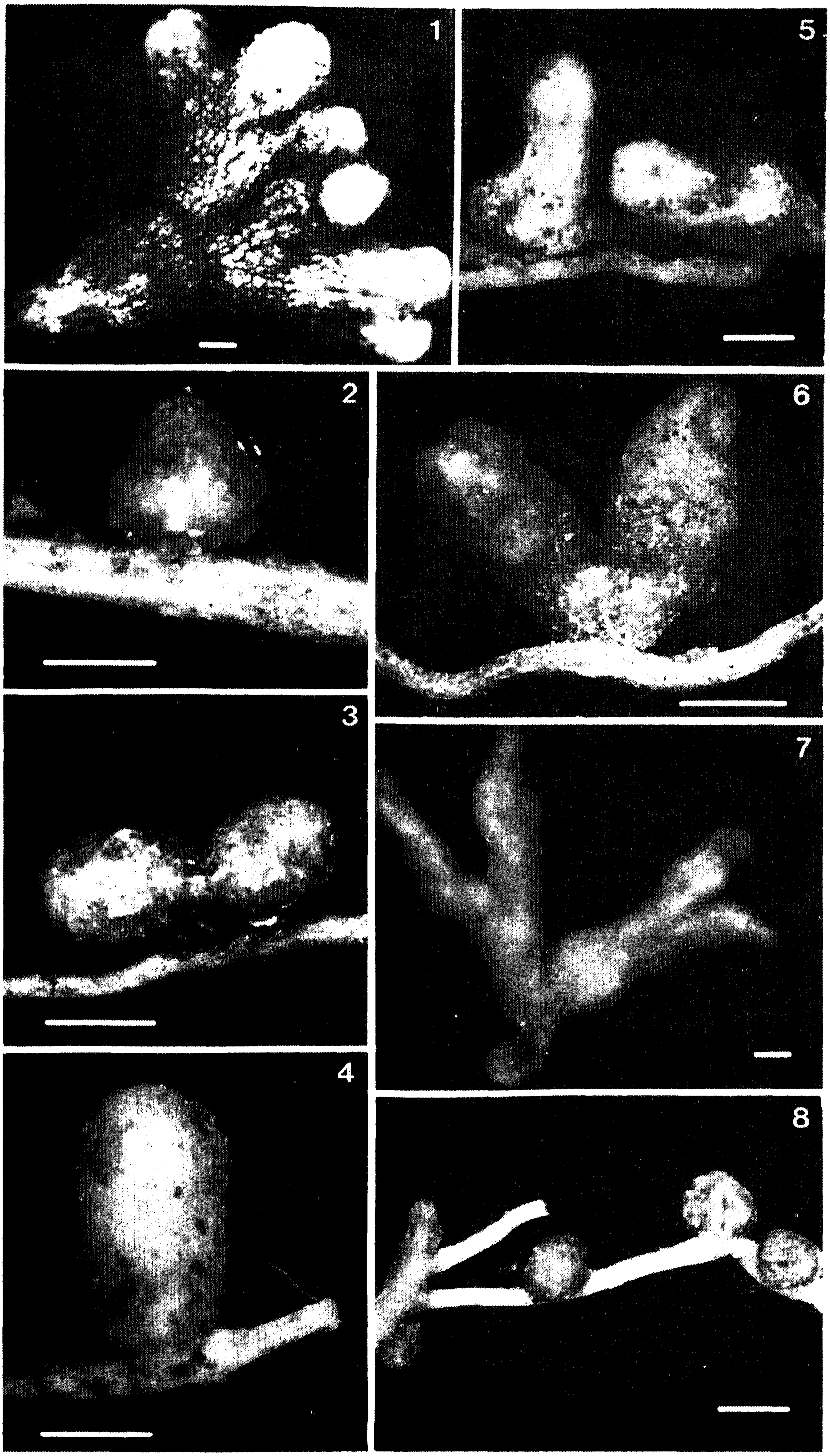
There is substantial variation in nodule shape and size among the taxa. *C. brevispina* has the largest nodules (~17 × 20 mm) among all the 100 legumes screened for nodule occurrence (figure 1); all other species have very small (<2 × 1 mm) to medium-sized nodules (~6 × 5 mm), except *Thermopsis* which has relatively larger nodules. Besides the medium-sized nodules, in *Argyrolobium flaccidum* and *Astragalus leucocephalus* the tip of secondary root and or rootlets often bear perennial branched cluster of very small nodules (with 10–50 very small nodules in each cluster) whenever the nodule forming roots failed to penetrate into the substratum because of its stony nature. Such coralloid nodules also contain leghaemoglobin. The coralloid nodules have been reported in the tribes Genisteae and Galegeae (Corby 1971). In fact, *Argyrolobium* belongs to the former tribe, whereas *Astragalus* is a member of the latter. The dimorphic nodules in *I. heterantha* (globose entire and globose bifurcate; figures 2 and 3), *A. leucocephalus* (elongate and branched nodules) and *Astragalus graveolens* (globose and elongate; figure 4) and the trimorphic ones (elongate, bifurcate and coralloid; figures 5–7) in *A. flaccidum* suggest that nodule shape and size are greatly influenced by the habitat factors and are of little taxonomic and evolutionary significance. However, nodule morphology has been used for assessing taxonomic and evolutionary affinities among leguminous taxa (Corby 1971; de Faria *et al* 1984).

The prevalence of nodules on secondary roots and or rootlets may be due to edaphic conditions. Such nodulation patterns may also be suggestive of infection through lateral root junction rather than via root hairs. In fact, Chandler (1978) and Chandler *et al* (1982) reported a similar mode of infection in two leguminous taxa (*Arachis* and *Stylosanthes*). The nodules observed on plants during the months of April–May were mostly dry and shrunken, whereas the nodules observed on plants during July–September were fresh and turgid. In fact, nodules were observed on plants of *Lespedeza* (figure 8) only during July–September. These observations suggest that nodulation chiefly occurs during July–September, the months in which maximum rainfall occurs. Consequently, it is essential to seed the legumes before the monsoon sets in, for the revegetation of marginal lands.

In *Thermopsis*, which has perennial, globose apical nodules, nodulation occurs at vegetative stage; in all other legumes investigated nodulation was observed at vegetative, flowering and fruiting stages.

The senescence of nodules after the monsoon and nodulation during monsoon coupled with perennial nodules demonstrate that the Himalayan legumes have evolved a unique adaptive strategy for the regulation of growth patterns: (i) the aerial growth essentially occurs only during June–September, a period characterised by ample sunshine and rainfall, and nodulation during this period meets the excess nitrogen demand for rapid growth; (ii) the perennial nodules enable the legume plants to fix atmospheric nitrogen even in the absence of fresh nodulation.

Of all the legumes, *I. heterantha* and *L. stenocarpa* are excellent fodder and firewood crops; *C. brevispina* is also used as a firewood and other legumes are effective soil binders (table 2). In fact, all the 7 new nodulating legumes investigated are useful in the revegetation of denuded Himalayan ecosystems.



Figures 1–8. Nodules of 5 legumes. **1.** *Caragana brevispina.* **2 and 3.** *Indigofera heterantha.* **4.** *Astragalus graveolens.* **5–7.** *Argyrolobium flaccidum.* **8.** *Lespedeza stenocarpa.* (Bar = 1 mm).

Acknowledgements

This work was supported by Department of Environment, Forests and Wildlife (Ministry of Environment and Forests, Government of India) grant No. J-12017/63/83 En. I. The help rendered by the Indian Army at Leh and the Eco-Development Task Force (Himalayan Coy.) at Mussoorie is gratefully acknowledged.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 1, February 1988, pp. 49–53.
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Plant age, temperature and free moisture in relation to susceptibility of clusterbean to *Xanthomonas campestris* pv. *cyamopsidis*

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MS received 22 April 1987; revised 2 December 1987

Abstract. Inoculation of 15–30 day clusterbean plants by *Xanthomonas campestris* pv. *cyamopsidis* resulted in death of many plants due to rotting of stem whereas in 60–75 day old plants infection was confined to foliage only. There was negative correlation between disease severity and host age. Both temperature and duration of free moisture on plant surface influenced disease severity. Infection occurred at 20–35°C, 30°C being optimum and increased with increased period of free moisture availability.

Keywords. Clusterbean; *Xanthomonas campestris* pv. *cyamopsidis*; susceptibility; plant age; temperature; free moisture.

1. Introduction

Clusterbean or guar (*Cyamopsis tetragonoloba* (L.) Taub.) is an industrial crop now due to gum content in its seed known as mannogalactan, which is useful in paper manufacture as excellent substitute for carob gum. India earns around 80 crore rupees foreign exchange annually by export of guar gum. Bacterial blight disease incited by *Xanthomonas campestris* pv. *cyamopsidis* (Patel, Dhande and Kulkarni). Dye has become a serious problem in many clusterbean production areas, during monsoon season i.e. July–October (Patel *et al* 1953; Srivastava and Rao 1963). Early infection may reduce the yield to a great extent (Gandhi and Chand 1985).

Earlier studies on epidemiology (Srivastava and Rao 1963) have only dealt with the environmental conditions favourable for bacterial blight development. Other factors such as host susceptibility, temperature and duration of leaf wetness are also important. In several other host pathogen systems, host susceptibility varied during plant growth (Lutrell *et al* 1974; Shukla *et al* 1975; Burr and Hurwitz 1981; Getz *et al* 1983) and influenced by temperature as well as leaf wetness (Wallin and Loonan 1971; Black and Neely 1978; Shaw 1986). The present investigation determined the age at which the clusterbean plants are most susceptible to infection and to study the effect of temperature and free moisture on disease occurrence so as to devise suitable control strategies for combating the losses caused by it.

2. Materials and methods

2.1 *Inoculum*

A virulent isolate was used in this study. Cultures were grown on yeast glucose chalk agar medium for 24 h at 30°C. Inoculum was prepared by washing cells from agar surface with sterile distilled water (SDW). Final inoculum concentration was adjusted by dilution with SDW to approximately 10^7 colony-forming units per ml as determined by standard turbidimetric and dilution plate techniques.

2.2 *Host age*

In the green house, seeds of two cultivars of clusterbean Pusa Navbahar (highly susceptible) and HG75 (moderately susceptible) were sown periodically in 30 cm earthern pots containing field soil so as to get plants of 15, 30, 45, 60 and 75 days age and 3 plants per pot. Each treatment was replicated thrice. The plants of different age groups were spray inoculated simultaneously during first week of August, when conditions are generally congenial for disease development in the field. Control plants were treated with SDW. Both inoculated and control plants were placed in muslin cloth covered chambers with periodic misting to keep them wet. After 48 h incubation period plants were removed from the chambers and placed on green house benches. Disease severity was recorded after 10, 20, 30 and 40 days of inoculation on 1–5 scale and using Mckinney's formula (1923).

2.3 *Temperature and duration of leaf wetness*

Twenty day old seedlings in 12 cm plastic pots, two in each pot and 3 pots of each cultivar per treatment were spray inoculated, covered with polythene bags wetted with SDW from inside and placed in temperature controlled chambers at 20, 25, 30 and 35°C. After 6, 12, 24 and 48 h pots were taken out from each chamber, bags removed, leaves dried with blotting paper and placed in the green house. Temperature in the green house fluctuated between 20°C (night) and 35°C (day).

3. Results and discussion

3.1 *Host age*

Disease index was maximum in 15 day old plants and decreased with increasing age of the plants (table 1). Plants of 15–30 days expressed symptoms on foliage and on

Table 1. Plant age in relation to development of bacterial blight in two clusterbean cultivars.

Plant age at inoculation (days)	Pusa Navbahar		HG 75	
	Disease[a] index (%)	Symptoms	Disease index (%)	Symptoms
15	87·77	Leaf spots, blight and rotting of stem	63·33	Leaf spots, blight and rotting of stem
30	62·00	–do–	46·66	–do–
45	40·00	Leaf spots, blight and streaks on stem	25·55	Spots and blight only
60	26·66	Leaf spots and blight only	15·50	–do–
75	18·33	–do–	13·33	–do–
LSD (P=0·05)	13·22		13·95	
Correlation coefficient (r)	−0·9784**		−0·9629**	

[a] Disease index after 40 days of inoculation.

stem resulting in rotting of stem and collapse of many plants. In 60–75 day plants symptoms appeared on foliage only and all the plants remained erect. Thus there was negative correlation between plant age and severity of bacterial blight. Disease level was low (figure 1) after 10 days of inoculation in all plants. Disease built up rapidly later in 15–30 day plants in both the cultivars and slowly in 45–75 day old plants.

Changes in host susceptibility with age have been recorded in other bacterial plant diseases. Chand and Walker (1964) observed higher multiplication of *Pseudomonas syringae* pv. *lachrymans* in young leaves than in old cucumber leaves. Coyne *et al* (1973) noted that bean plants were more susceptible to *X. campestris* pv. *phaseoli* when in reproductive stage than in vegetative state. The high susceptibility of young

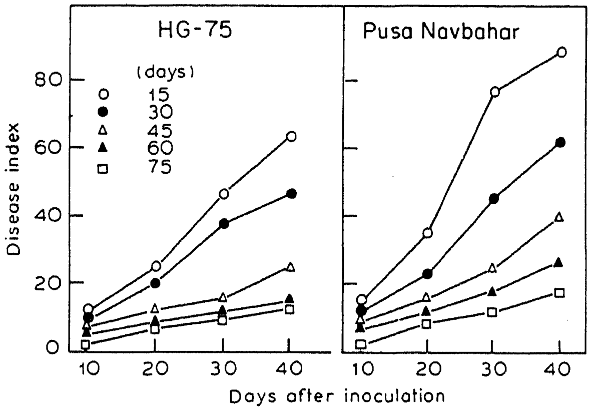

Figure 1. Progress of bacterial blight in clusterbean plants of different age groups.

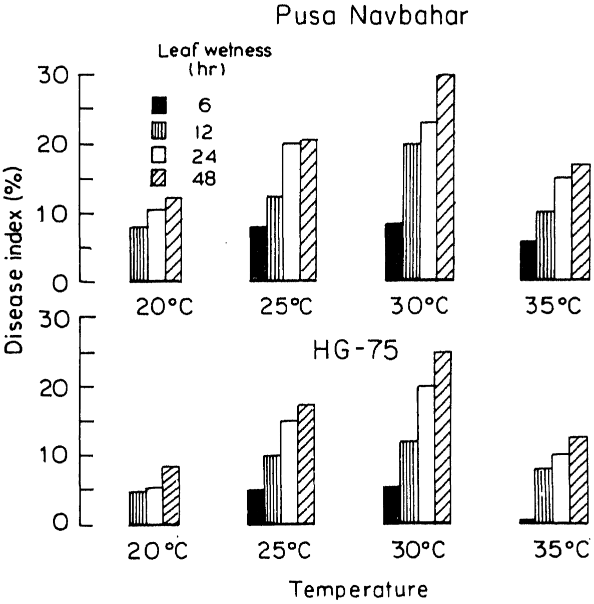

Figure 2. Interaction of temperature and leaf wetness duration on the development of bacterial blight of clusterbean.

plants observed in the present studies may be attributed to more succulence of plant parts and proneness to infection due to greater water congestion in wetted foliage. A positive correlation between water congestion in intercellular spaces and infection by *P. syringae* pv. *tabaci* has been observed (Johnson 1947). Also, active metabolism in young plants might provide easy access to nutrients required for growth and multiplication of the pathogen. Hence plant development stage must be considered while selecting resistant genotypes and in adopting disease management measures.

3.2 *Temperature and free moisture*

At 20°C when free moisture remained on leaves for 6 h, infection did not occur in both the cultivars and only traces of blight were seen in HG 75 at 35°C for this duration of leaf wetness (figure 2). When free moisture was available for more than 6 h, symptoms appeared on plants incubated at the 4 temperatures and varied with temperature. Maximum infection occurred when free moisture was available for 48 h.

Table 2. Mean effects of leaf wetness duration and temperature on intensity of bacterial blight of HG 75.

Leaf wetness duration (h)	Temperature (°C)				Mean disease intensity (%)
	20	25	30	35	
6	0·00 (1·00)	4·66 (2·38)	5·50 (2·54)	0·00 (1·00)	2·54 (1·73)
12	5·00 (2·45)	10·00 (3·26)	13·33 (3·77)	8·00 (2·99)	9·08 (3·12)
24	5·33 (2·52)	15·00 (3·99)	20·00 (4·56)	10·25 (3·35)	12·65 (3·60)
48	8·50 (3·07)	17·50 (4·29)	25·00 (5·10)	12·50 (3·66)	15·88 (4·03)
Mean disease intensity	4·71 (2·26)	11·79 (3·48)	15·96 (3·99)	7·69 (2·75)	
	Temperature (T)	Leaf wetness (W)	T × W		
SEM ±	(0·10)	(0·10)	(0·22)		
LSD ($P=0·05$)	(0·20)	(0·20)	(0·46)		
LSD ($P=0·01$)	(0·29)	(0·29)	N.S.		

Numbers in parentheses are average of $\sqrt{n+1}$ transformed data.

Table 3. Mean effects of leaf wetness duration and temperature on intensity of bacterial blight of Pusa Navbahar.

Leaf wetness duration (h)	Temperature (°C)				Mean disease intensity (%)
	20	25	30	35	
6	0·00 (1·00)	8·00 (3·00)	8·50 (3·07)	6·33 (2·70)	5·70 (2·44)
12	8·00 (2·99)	12·50 (3·67)	20·25 (4·62)	10·00 (3·31)	12·68 (3·65)
24	10·50 (3·39)	20·00 (4·57)	22·85 (4·85)	15·00 (3·97)	17·09 (4·19)
48	12·33 (3·64)	20·50 (4·62)	30·00 (5·56)	16·66 (4·15)	19·89 (4·49)
Mean disease intensity	7·71 (2·76)	15·26 (3·92)	20·31 (4·52)	11·92 (3·53)	
	Temperature (T)	Wetness (W)	T × W		
SEM ±	(0·13)	(0·13)	(0·30)		
LSD ($P=0·05$)	(0·27)	(0·27)	(0·61)		
LSD ($P=0·01$)	(0·37)	(0·37)	N.S.		

Numbers in parentheses are average of $\sqrt{n+1}$ transformed data.

At 30°C disease index was highest followed by 25, 35 and 20°C in both the cultivars. In HG 75 (table 2) significant differences were observed between all the temperatures and leaf wetness durations in relation to disease index. Interaction between free moisture duration and temperature was not significant at 20 and 35°C with 12 and 24 h leaf wetness. In Pusa Navbahar (table 3), significant differences in disease index were observed in all treatments except at 20 and 30°C with 12 and 24 h leaf wetness and at 20, 25 and 30°C with 24 and 48 h leaf wetness.

Thus, both temperature and duration of leaf wetness had significant effect in the development of bacterial blight of clusterbean. Temperature 25–35°C supported high level of infection and 30°C appeared to be the optimum, when free moisture was available for more than 6 h. The meteorological conditions which result in moderate temperature and adequate free moisture will enhance the possibility of infection under natural conditions in the field.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 1, February 1988, pp. 55–59.
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Vesicular-arbuscular mycorrhizal associations of sesamum

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MS received 4 April 1987; revised 14 November 1987

Abstract. Extensive mycorrhizal infection of sesamum under field conditions was observed. The plants were found to be mycorrhizal even in the early stages. There was a progressive increase in mycorrhizal infection up to 6 weeks, then declined slightly. A gradual increase in vesicle count per 1 cm segment as the plants aged was noticed. Species of *Glomus*, *Gigaspora* and *Sclerocystis* were found in the rhizosphere soil. Among the spore types recorded, chlamydospores of *Glomus*, especially *Glomus mosseae* were predominant.

Keywords. *Sesamum indicum*; vesicular-arbuscular mycorrhizae; spores; sporocarps; *Glomus* spp.; *Gigaspora* spp.; *Sclerocystis pakistanica.*

1. Introduction

Vesicular-arbuscular mycorrhizae (VAM) are known to increase phosphorus uptake and growth in a number of agricultural crops, especially in soils low in available phosphorus (Gerdemann 1975; Hayman 1980). VAM can also improve uptake of copper (Timmer and Leyden 1980), zinc (Lambert *et al* 1979) and sulphur (Gray and Gerdemann 1973). Plants infected with mycorrhizal fungi exhibit significant yield increases over non-mycorrhizal plants (Khan 1975; Saif and Khan 1977). As there were no studies of mycorrhizas on sesamum (*Sesamum indicum* L.), the identity of the fungal partners, their development in root system and their spore count in corresponding rhizosphere were investigated.

2. Materials and methods

The sesamum crop was planted in small field plots during 1986 rabi (January–March). As the rabi season was warm and dry, the crop was grown under irrigation. The soil in which plants raised was a red laterite with pH 7·2, organic matter 0·28% and MHC 20·8%, and is known to harbour VAM fungi (Parvathi *et al* 1984; Vijayalakshmi and Rao 1987).

At 15-day intervals from the time of sowing until harvest (after 75 days), the plants were removed at random, their roots were excised, washed carefully, cut into 1 cm segments, cleared, stained and examined for VAM fungi (Phillips and Hayman 1970). The presence or absence of the fungus and the number of vesicles in 50 root segments of 1 cm length collected at random from a total of 5 plants were estimated on each occasion. The percentage of mycorrhizal infection was calculated as follows:

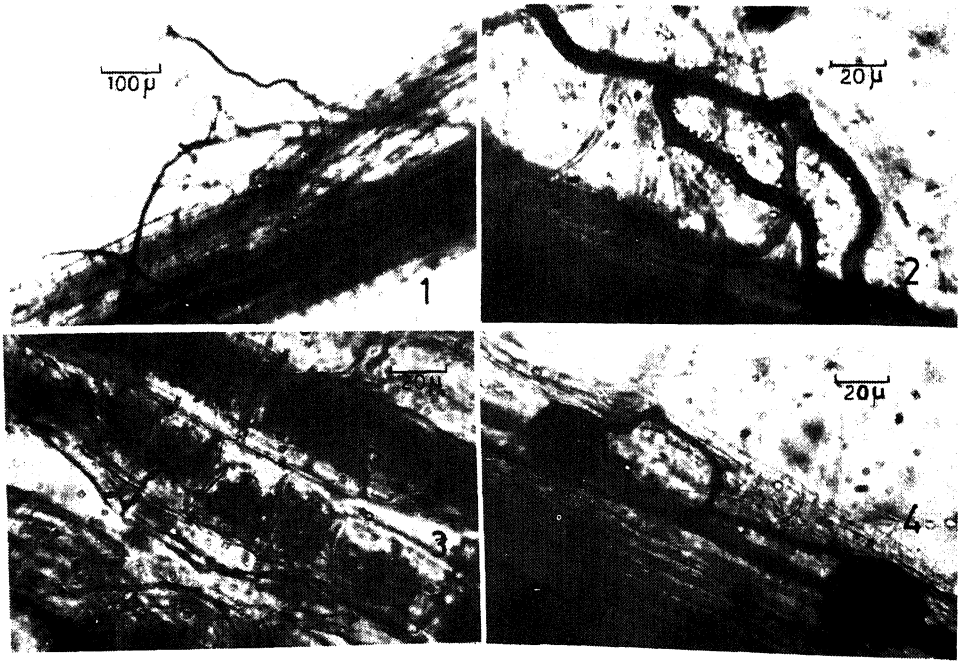
$$\text{VAM infection}(\%) = \frac{\text{No. of infected segments}}{\text{Total no. of root segments examined}} \times 100.$$

Spore types of VAM fungi and their abundance in rhizosphere were recorded simultaneously with root infection for 5 replicates, after extraction by the wet-sieving and decanting technique (Gerdemann and Nicolson 1963).

3. Results

Mycorrhizal infection was mostly confined to finer lateral roots in the initial stages. Profuse external hyphae with prominent angular projections were common on the root surface (figure 1). Entry was mostly through the epidermal cells (figure 2). Mycorrhizal infection with internal hyphae, arbuscules (figure 3) and vesicles (figure 4) could be seen in 15-day old plants. The per cent root segments infected by VAM fungi increased progressively until 6 weeks (table 1), later there was an apparent decline which may be due to the absence of infection in newly formed roots. The incidence of arbuscules in root segments was initially high but declined slightly at later stages. Vesicle count per 1 cm segment progressively increased with increasing plant age.

A progressive increase in spore population in rhizosphere soil as the plants aged was found. Chlamydospores of *Glomus* species were predominant among the spore types recorded. Of the 3 species of *Glomus* identified viz. *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe, *Glomus macrocarpum* Tul. and Tul. (figure 5) and *Glomus multicaule* Gerdemann and Bakshi (figure 6), *G. mosseae* was quite abundant in soil samples. Sporocarps of *Sclerocystis pakistanica* Iqbal and Bushra (figures 7 and 8) were also found but not abundant. These VAM fungi were identified based on the descriptions given by Mosse and Bowen (1968), Gerdemann and Trappe (1974), El-

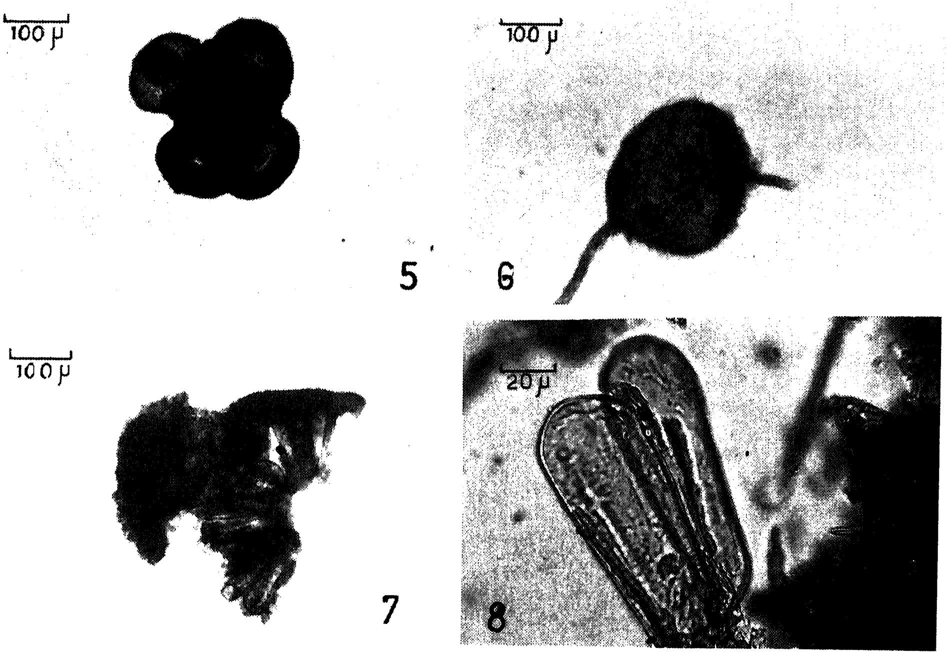


Figures 1–4. **1.** External mycelium over root surface. **2.** Entry of VAM fungi into the root. **3.** Arbuscules in root cortex. **4.** Vesicles/spores in root cortex.

Table 1. Mycorrhizal development in *S. indicum*.

Plant age (days)	Root colonization[a]		Average no. of internal vesicles cm^{-1} root	Spores and sporocarps in rhizosphere soil (50 g)			
	External	Internal		*Glomus* spp.	*Gigaspora* spp.	*S. pakistanica*[b]	Unidentified spore types
15	39·1 (5·4)	42·5 (4·9)	8·2 (1·9)	45 (15·0)	9 (1·6)	—	15 (4·3)
30	50·0 (7·2)	56·8 (6·5)	12·5 (2·8)	87 (12·0)	10 (3·2)	1 (0·6)	41 (8·5)
45	63·3 (11·1)	48·3 (9·8)	19·1 (2·1)	115 (14·5)	15 (4·1)	1 (0·6)	44 (7·9)
60	49·7 (12·0)	36·8 (14·2)	19·9 (3·6)	208 (21·8)	18 (2·0)	2 (1·6)	39 (6·4)
75	25·6 (9·3)	30·0 (7·8)	20·5 (2·1)	239 (28·6)	20 (5·0)	2 (1·4)	44 (6·8)

[a]Per cent root segments examined. [b]Sporocarps.
Numbers in parentheses denote SD values (n = 5).



Figures 5–8. 5. Sporocarp of *G. macrocarpum*. 6. Chlamydospore of *G. multicaule*. 7. Radial arrangement of spores around central plexus of hyphae in *S. pakistanica*. 8. Chlamydospores of *S. pakistanica*.

Giahmi *et al* (1976), Gerdemann and Bakshi (1976) and Iqbal and Perveen (1982). Besides the above mentioned spore types, azygospores of two species of *Gigaspora* were isolated, but not in sufficient numbers for confirming the identification.

4. Discussion

This study confirms the formation of VA mycorrhizas in sesamum in a tropical soil in India. The roots were found to be mycorrhizal even in early stages, in contrast to other hosts like groundnut, pigeon pea and black gram in the same soil (Rao and Parvathi 1982). As reported by Rao and Parvathi (1982), an apparent decline in root infection by VAM fungi as the plants aged in the above crops was also noticed in sesamum and the cause for the decline in root infection by VAM fungi needs investigation.

The extensive occurrence of internal vesicles with simple or funnel shaped attachments indicates that the VAM fungus may be a species of *Glomus*. This observation is confirmed by the predominant occurrence of chlamydospores of *G. mosseae* and *G. macrocarpum* in the rhizosphere soil. Earlier studies suggest that some species of VAM fungi were more efficient in phosphorus uptake than others (Jensen 1982; Krishna and Dart 1984; Schubert and Hayman 1986). The present study indicated the occurrence of a wide variety of endophytes in a tropical red lateritic soil and attempts are now being made to obtain monoxenic cultures for determining their efficacy on nutrient uptake of this important oil yielding crop.

Acknowledgements

The authors wish to thank Dr I R Hall, Invermay Agricultural Research Centre, New Zealand for providing literature on the taxonomy of Endogonaceae. One of the authors (MV) is grateful to the Council of Scientific and Industrial Research, New Delhi for financial assistance.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 1, February 1988, pp. 61–66.
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Effect of some phenolic acids on quality and yield of chickpea seeds

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Abstract. Exogenous application of 1-amino; 4-sulphonate, β-naphthol; 8-amino; 3–6 disulphonate, γ-naphthol; 1, 3-dihydroxybenzene and 4, 6-Bis (diazonaphthalene sulphonate-resorcinol) (5, 20, 50 ppm) increased number of pods per plant, number of seeds/pod and improved the yield (kg/ha) in chickpea. Some of the compounds enhanced total free amino acids and soluble protein and total soluble sugars. The effects of the phenolic acids on growth and development were independent of their structural configuration.

Keywords. Phenolic compounds; yield; quality; *Cicer arietinum*.

1. Introduction

India produces 74·58% of the total world production of chickpea, and has 75·52% of the total world acreage under chickpea (FAO 1983). The average yields of chickpea in India are very low and often uncertain due to pathological and physiological constraints. The use of plant growth regulators in general and phenolic compounds in particular in regulating morphogenetic differentiation and alleviating various physiological disorders causative of low and uncertain yields in different crop plants is well documented (Nickel 1983; Malik *et al* 1986). Besides quantitative increase, there is also the need for enhancing the nutritional value of chickpeas. Malik *et al* (1986) reported that 4 phenolic compounds, viz. H-acid, 1, 2, 4-acid, resorcinol and RD-brown, were highly effective in improving yield and nutritional quality of some peanut varieties. The present investigation was carried out to determine the effects of some low-cost phenolic acids on quality and yield of mature chickpea seeds.

2. Material and methods

Chickpea (*Cicer arietinum* L.) (GL-769) was sown in the field area of the Botany Department during 1983 with 3 replications of each treatment having a plot size of 4·5 × 3 m. The crop was raised according to the recommended practices for fertilizers, irrigation and plant protection measures (package of practices, 1983, PAU, Ludhiana). Two sprays were given—one at the time of flower initiation, i.e. 90 days after sowing and the second after 15 days of first spray of 5, 20, 50 ppm concentrations of different phenolic compounds (figure 1). Controls consisted of spraying with distilled water alone.

The effect of phenols on yield contributing parameters was computed on the basis of the following parameters recorded at the harvest time from 5 randomly selected plants from each treatment. The data on number of pods/plant at harvest time; number of seeds/pod taken from 10 pods from 5 randomly selected plants; 1000-seed weight selected after harvesting from each replication recorded in grams; and grain yield recorded from net area harvested and expressed as kilograms per hectare were

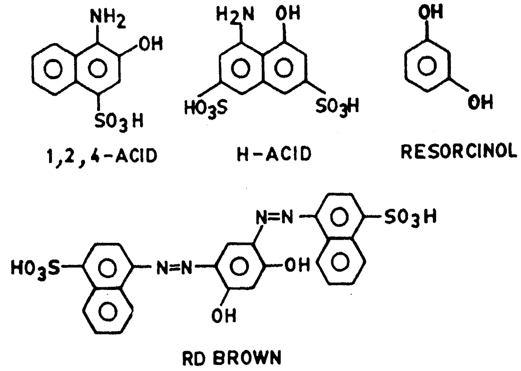

Figure 1. Phenolic compounds.

computed. Experimental plots were sprayed with a foot-sprayer of 400 l of solution per hectare. Five plants were selected from each of 3 replicated plots at harvest time for recording the data.

For ascertaining the quality of seeds at harvest time (49 days after anthesis), several biochemical parameters of mature whole seeds were analyzed according to standard spectrometric techniques, e.g. total soluble proteins (Lowry *et al* 1951); total free amino acids (Lee and Takahashi 1966) and starch and total soluble sugars (Loews 1952) were estimated.

For every estimation, 15 mg of dry material was used and a mean of 3 replicate observations was recorded. The details of phenolic acids used are given in table 1. Solutions of the acids were prepared by dissolving in a few drops of ethanol and then the final volume was adjusted with water.

3. Results and discussion

The data given in table 2 indicate significant differences between the controls and treatments in regard to both qualitative as well as yield contributing parameters. Table 2 shows that all the phenolic acids at all concentrations used significantly increased pod number per plant, except 5 ppm of RD-brown and resorcinol. The highest concentration (50 ppm) was most effective, except in resorcinol where 20 ppm gave the maximum number of pods. The data in table 2 shows that higher concentration (50 ppm) increased the number of seeds per pod in all the phenolic acids but the increase was non-significant. Table 2 also indicates non-significant differences among different treatments for 1000 seed weight. All the phenolic acids, at all the tried concentrations, improved the yield level significantly. On the whole, H-acid (20, 50 ppm) and 1, 2, 4-acid (50 ppm) showed the highest increment in yield compared with all other treatments.

Compared with the controls, plants sprayed with H-acid, resorcinol (5 ppm), RD-brown (5 ppm) and 1, 2, 4-acid (50 ppm) had increased content of free amino acids in their seeds. H-Acid (5 and 20 ppm), resorcinol (5, 20, 50 ppm) and RD-brown (5 and 20 ppm) significantly increased the protein content in the seed (table 3). Among the 3 phenols, RD-brown (5 ppm) was most effective. Compared with phenolic acid treatments [(1, 2, 4-acid; H-acid, resorcinol (5, 20, 50 ppm) and RD-brown (5 and 20 ppm)], the seeds of control plants had lower contents of sugars (mg g^{-1} dry

Table 1. Chemistry of 4 compounds used.

Chemical name	Common name	Concentration used (ppm)	Molecular weight
Monophenols			
1-Amino; 4-sulphonate, β-naphthol	1, 2, 4-acid	5, 20, 50	239
8-Amino; 3 6 disulphonate, γ-naphthol	H-acid	5, 20, 50	319
Diphenols			
1, 3-Dihydroxy benzene	Resorcinol	5, 20, 50	710
4, 6-Bis (Diazonaphthalene sulphonate-resorcinol)	RD-Brown	5, 20, 50	622

Table 2. Effect of phenols on yield contributing parameters in chickpea.

Treatment (ppm)		Number of pods/plant	Number of seeds/pod	Thousand seed wt. (g)	Yield kg/hectare
Control		44·3	2·6	144·07	1185
1, 2, 4-Acid	5	48·6	2·2	174·21	1445
	20	50·2	2·9	165·88	1659
	50	55·4	3·1	151·11	1908
H-Acid	5	46·5	2·5	148·85	1460
	20	60·2	1·8	169·66	1979
	50	68·4	3·4	180·71	2015
Resorcinol	5	39·3	1·5	158·33	1659
	20	62·5	2·6	153·13	1397
	50	47·1	2·9	161·11	1485
RD-Brown	5	42·5	2·7	164·58	1266
	20	53·8	3·4	162·16	1604
	50	57·6	3·3	179·03	1815
CD at 5%		2·71	NS	NS	6·85

weight). Resorcinol (50 ppm) recorded the highest content of soluble sugars in the seeds. On the contrary, starch content of seeds decreased in most treatments, except H-acid (20 ppm), resorcinol (5 ppm) and RD-brown (20 and 50 ppm). The maximum increase was noticed with RD-brown (50 ppm).

The phenolic acids had a promoting effect on yield per unit area. Yield (kg per hectare) was notably high in 1, 2, 4-acid (50 ppm), H-acid (20 and 50 ppm) and RD-brown (50 ppm) treatments. Correspondingly, the average seed weight (1000-seed weight) was also higher in all the treatments than in control. Singh *et al* (1980) used α- and β-naphthol, salicylic acid, caffeic acid in chickpea and noticed increase in flowers/plant and number of pods per plant. Nanda *et al* (1976) reported the involvement of different phenols and their synergistic effect with gibberellic acid on flowering. Notably, 1, 2, 4-acid (5 ppm), H-acid (50 ppm) and RD-brown (50 ppm) were highly promotive. The average number of seeds per pod was promoted by all the treatments and increased with increasing concentrations, except with H-acid (20 ppm), resorcinol (5 ppm) and 1, 2, 4-acid (5 ppm). The number of pods per plant was enhanced with increasing concentration of all the substances; of these, H-acid (50 ppm) was most effective. Clearly, increase in yield by phenolic acids was quite

Table 3. Effect of phenols on some biochemical (seed quality mg/g dry weight) parameters in mature chickpea seeds.

Treatment (ppm)		Soluble proteins	Free amino acids	Starch	Soluble sugars
Control		144·69	15·02	175·5	28·0
1, 2, 4-Acid	5	120·02	11·54	165·0	38·5
	20	144·71	18·40	140·0	44·0
	50	129·00	26·15	135·0	38·0
H-Acid	5	168·67	23·30	165·0	39·0
	20	169·39	30·21	230·0	28·0
	50	145·40	35·90	110·0	30·5
Resorcinol	5	157·41	20·02	245·5	36·5
	20	188·09	13·35	125·0	26·5
	50	199·73	12·84	180·5	48·0
RD-Brown	5	219·02	25·68	180·5	35·0
	20	168·00	15·35	270·0	40·0
	50	127·09	13·21	245·0	24·0

pronounced, especially with H-acid and 1, 2, 4-acid. Present investigation supports the studies of Datta *et al* (1977), Nanda and Kumar (1977), Datta *et al* (1978), Tayal and Sharma (1980) and Malik *et al* (1986) with different crop species. Similarly, through exogenous application of some phenols, Singh *et al* (1980) obtained increased economic yields of chickpea. Enhancement of yield parameters in mungbean (Chander 1982), peanut (Parmar *et al* 1982; Malik *et al* 1986), pigeon pea (Singh *et al* 1986) and rice (Parmar *et al* 1986) have been recorded.

A better nutritional quality of chickpea seed would depend upon the presence of high soluble protein, free amino acids and soluble sugar contents. On the contrary, high starch and low protein seeds are not nutritionally desirable. Among the treatments tried, H-acid (5 and 20 ppm), resorcinol (50 ppm) and RD-brown (5 ppm) produced better quality seeds. Amongst the yield contributing parameters, H-acid (20 and 50 ppm), RD-brown (50 ppm) and 1, 2, 4-acid (50 ppm) caused the best performance. The treatments which caused both qualitative and quantitative increments were H-acid (20 and 50 ppm), resorcinol (50 ppm) and RD-brown (5 ppm).

Total free amino acids and soluble sugar contents of mature seeds were enhanced by all the treatments, though RD-brown (50 ppm) caused retrogression of total soluble sugar content of the mature seeds. The treatments caused varied effects on the starch content of seeds compared with the control. All the concentrations of 1, 2, 4-acid, H-acid, except (20 ppm) and resorcinol (except 5 ppm) decreased starch content of the seeds, while all concentrations of RD-brown promoted its level. Monophenolics cause the activation of IAA-oxidase activity thereby lowering the auxin level (Henderson and Nistch 1962), and altering the source-sink relation. Wynn and Fore (1965) and Emmanual (1968) described phenols as the most effective inhibitors of oxidative phosphorylation. Phenols promote the biosynthesis of nucleic acids and proteins (Kefeli and Kutacek 1977), also increase the activities of amylases, IAA-oxidase, peroxidase and polyphenol oxidase (Kumar and Nanda 1981). Phenolics are also reported to increase seed protein content in mungbean (Kalita and Shah 1983) and oil in peanut (Malik *et al* 1986).

Among the phenolic acids, 1, 2, 4-acid and H-acid are monophenols, whereas resorcinol and 4, 6-bis (diazonaphthalene sulphonate)-resorcinol are diphenols. Both ne monophenols significantly increased number of pods/plant and yield (kg/ha) ver the control. The physiological role of phenolic substance is controversial. Some orkers cast doubt on their role in the regulation of plant growth since they are onfined to plant vacuoles. Kefeli and Dashek (1984) have inferred that endogenous henols are stimulatory and promote growth. These workers have suggested that affeic acid and ferulic acid have auxin-like characteristics. The general view is that ionophenols act as cofactors of IAA oxidase, whereas polyphenols inhibit the ctivity of IAA oxidase leading to the synergism of IAA action (Tomazewski and himann 1966). Hess (1968) suggested that the occurrence of two-OH groups at the *'tho*-position with a free *para*-position was an essential requirement for a phenolic ompound to be biologically active. Pilet (1966) and Akburg and Johanson (1969) oticed that di- and polyphenols were promotive. A lack of structural relationship of henols with their activity is reported recently (see Malik *et al* 1986). The present udies clearly show that phenolics could be effectively used in increasing yield of ulses.

Phenolic acids, thus, make available high levels of soluble sugars by suppressing xidative phosphorylation which is detoured towards biosynthesis of free amino cids and protein. Our studies also show that increased free amino acids content is ot due to enhanced protein degradation but rather due to their increased roduction and interconversion. This may be the cause for high levels of soluble roteins, free amino acids and soluble sugars recorded in the present investigation.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 2, April 1988, pp. 67–69.

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A new species of *Spegazzinia* from Western Australia

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Abstract. Hyphomycete collected on *Xanthorrhoea preissii* Endl. (Xanthorrhoeaceae) in western Australia is described as a new species of *Spegazzinia* Sacc., *Spegazzinia xanthorrhoeae*.

Keywords. Hyphomycete; *Spegazzinia*; fungi on *Xanthorrhoea*.

In the course of a study of microfungi from Western Australia, an interesting hyphomycete was collected on dead leaf base of *Xanthorrhoea preissii* Endl. (Xanthorrhoeaceae). A description of the fungus is given below (figure 1).

The fungus forms effuse, black, shiny colonies on the substratum. No mycelium has been seen and the colonies consist almost entirely of a basal stratum of conidiophore mother cells from which conidiophores and conidia are produced, the whole forming a sporodochium. The conidiophore mother cells are hyaline, variable in shape and 3·7–9·6 × 2·1–4·8 μm (mean 7 × 3·7 μm). The conidiophores are basauxic, simple, hyaline to subhyaline, and 6–22 × 1·4–3·7 μm. The conidia are solitary, acrogenous, dry, ellipsoidal, brown to dark brown, verrucose, muriform, with 3 transverse septa, slightly constricted at septa, with longitudinal septa in one or both middle cells, with the two end cells often paler in colour than the middle cells and 22–30 × 11–18 μm. The apical cell is rounded, and the basal cell carries at its base the broken part of the upper portion of the wall of the conidiophore mother cell as a frill corresponding to the broken lower part which remains a cupulate structure.

In the development of the conidium, the conidiophore arises from a conidiophore mother cell, the conidium initial developing as a swelling from a locus thereon. The conidium initial is hyaline to subhyaline and becomes verrucose very early. In turn, it becomes honey- or golden-coloured and develops septa one by one as it enlarges. In the meantime the conidiophore grows basauxically and, in the process, the outer wall of the conidiophore mother cell is broken. Part of the broken outer wall of the conidiophore mother cell now remains as a frill at the base of the conidium that is pushed up by the elongating conidiophore.

The fungus has been brought into culture. In culture the vegetative hyphae are hyaline, septate, branched and 2·9–4·4 μm wide. The conidiophore mother cells are 5–14 μm long and 3·7–6 μm wide. The conidiophores appear to be shorter than in the natural material: they are 8–21 × 2·5–2·9 μm. The conidia are 18–31 × 10–16 μm.

The simple basauxic conidiophores produced from conidiophore mother cells and the solitary, acrogenous, muriform conidia suggest the fungus may be accommodated in the genus *Spegazzinia* Sacc. The fungus is quite distinct from species of *Spegazzinia* so far known and is here named *Spegazzinia xanthorrhoeae*.

Spegazzinia xanthorrhoeae Subramanian sp. nov.

Coloniae effusae, nigrae nitidae. Forma matricellularum conidiophororum variabilis,

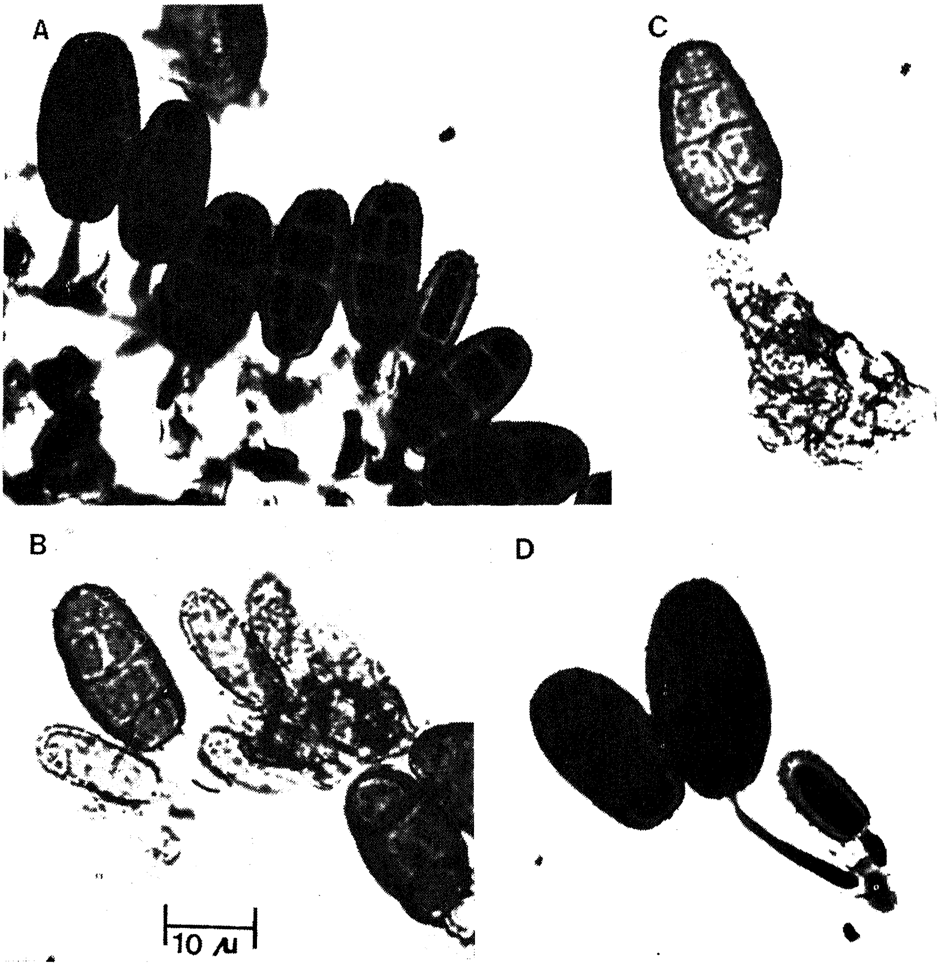

Figure 1. *S. xanthorrhoeae* from type material CVS 5a. **A.** Conidiophore mother cells, conidiophores and conidia. **B.** Conidia in various stages of development. **C.** Mature conidium still attached to the basauxic conidiophore. **D.** A young developing conidium and a mature conidium on a basauxic conidiophore.

hyalina, 5–14 × 3·7–6 μm. Conidiophora simplices, basauxica, hyalina vel subhyalina, usque ad 25 μm longa, 3·7 μm lata. Conidia solitaria, ellipsoidea, verrucosa, brunnea, plerumque tribus septis transversis instructa, 1 vel 2 cellularum mediarum longitudinaliter septatis, ad septa modice constricta; cellulis extremis saepe magis pallidioribus quam cellulis mediis, 22–30 × 11–18 μm. Typus in basi foliorum emortuorum e *Xanthorrhoea preissii* Endl. (Xanthorrhoeaceae), Mandurah 20 km meridieum versus Western Australia, 23 August 1987, No. 5a, leg. C V Subramanian.

Colonies effuse, black, shiny. Conidiophore mother cells variable in shape, hyaline, 5–14 × 3·7–6·0 μm. Conidiophores simple, basauxic, hyaline to subhyaline, up to 25 μm long, 3·7 μm wide. Conidia solitary, ellipsoidal, verrucose, brown, mostly with 3 transverse septa, slightly constricted at septa, with longitudinal septa in one or both middle cells, with the two end cells often paler in colour than the middle cells 22 30 × 11 18 μm.

Type: On dead leaf base of *Xanthorrhoea preissii* Endl. (Xanthorrhoeaceae), 20 km south of Mandurah, Western Australia, 23 August 1987, No. 5a, Coll. C V Subramanian

Other collections: On dead inflorescence stalk of *Xanthorrhoea preissii*, John Forrest National Park, Darling Range, WA, 27.8.1987. Coll. C V Subramanian (Nos 9a, 10a) and Madong Reserve, Swan Coastal Plain, WA, 2.9.1987. Coll. Giles Hardy (No. 16).

Culture: Isolated by Giles Hardy from the type specimen No. 5a listed above.

The author is thankful to the University of Western Australia for a visiting fellowship during the tenure of which this work was carried out. Thanks are also du to Prof. Alan Robson and Dr K Sivasithamparam for the facilities, Mr Giles Hard for technical assistance and Dr Wilson of the West Australian Herbarium fo rendering the diagnosis of the new species into latin.

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 2, April 1988, pp. 71–88.
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Uptake and assimilation of nitrogen by marine diatoms—I. Kinetics of nitrogen uptake

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MS received 16 April 1987; revised 12 March 1988

Abstract. Axenic cultures of marine diatoms, *Achnanthes hauckiana* Grun., *Amphora coffeaeformis* (Agardh) Kütz., *Fragilaria pinnata* Ehr., *Synedra tabulata* (Agardh) Kütz., *Thalassiosira weissflogii* (Grun.) Fryxell et Hasle (= *Thalassiosira fluviatilis* Hustedt) and *Triceratium dubium* Brightwell isolated from tropical waters were used for a comparative study of kinetics of NO_3^-, NO_2^- and NH_4^+ uptake. Nitrogen uptake by diatoms in this study followed the classical kinetics reported by previous workers. Diatoms which showed high K_s for NO_3^- uptake had high K_s for NH_4^+ and NO_2^- uptake. But no correlation could be obtained between cell size and K_s for nutrient uptake and an inverse relationship between specific growth rate and K_s was also not obtained. However, there was good correlation between the observed K_s and nutrient status of the habitats from which the diatoms were isolated. Studies on the interactions between NO_3^-, NO_2^- and NH_4^+ revealed that NH_4^+ inhibited both NO_2^- and NO_3^- uptake non-competitively whereas NO_3^- and NO_2^- inhibited each other in a competitive manner. Inhibition by NO_3^- of NO_2^- uptake was greater than the reverse situation.

Keywords. Kinetics of nitrogen uptake; marine diatoms; nitrogen assimilation.

1. Introduction

Production of phytoplankton greatly depends upon the available nutrients, especially nitrogen. Various species of diatoms exhibit different levels of efficacy to take up and assimilate high or low levels of nutrients. Growth of those species which can most efficiently assimilate and utilize low concentrations has a great advantage in nutrient poor waters. For this reason there is currently a great interest in understanding the relationship between kinetics of uptake of nutrients by diatoms and nutrient levels (Bonin *et al* 1986).

Probably the first investigation on the rate of nutrient uptake by a marine phytoplankton species was conducted by Ketchum (1939) using cultures of *Nitzschia closterium* (= *Phaeodactylum tricornutum* Bohlin). Rate of nitrate uptake measured over a short period was shown by him to vary hyperbolically with nitrate concentration in the medium, increasing rapidly with rising concentrations at low nitrate levels, then less quickly at higher levels. This hyperbolic relationship is typical of the Michaelis-Menten expression for the rate of an enzyme catalyzed reaction (Dugdale 1967; Eppley and Coatsworth 1968; MacIsaac and Dugdale 1969). Half saturation constants i.e. the concentration supporting half the maximum uptake rate, can be calculated for the hyperbolas using the Michaelis-Menten equation, $V = V_{\max} S/(K_s + S)$ where V is the rate and S the concentration. The constant K_s reflects the relative ability of phytoplankters to use low levels of nutrients and this may be of ecological significance (Eppley *et al* 1969). Another characteristic of the uptake hyperbolas, the maximum velocity ($V_{\max}$) is also of value for ecological interpretation (Dugdale 1967). V_{max} is taken as the maximum specific growth rate of the phytoplankton imposed by external conditions and is thus a variable, subject to effects of irradiance, day-length and temperature as well as specific properties of the phytoplankton (Eppley *et al* 1969; Bonin *et al* 1986). Calculations of these constants have proved useful for understanding marine phytoplankton production in upwelling regions (Dugdale and MacIsaac 1971), in oligotrophic area (MacIsaac and Dugdale 1972) and in coastal sewage outfalls (Dugdale and Whitledge 1970; Caperon *et al* 1971) and have provided an insight as to why certain phytoplankton assemblages are found in nutrient rich or nutrient poor waters (Eppley and Renger 1974). Eppley and Coatsworth (1968) have put forward the hypothesis that K_s for uptake of NO_3^-, NO_2^- and NH_4^+ for species of phytoplankton that occur in sequence in a seasonal succession should follow in order of declining nutrient concentrations. In other words, if the declining level of nutrient is important in species succession, each succeeding species should show a lower K_s than the preceding one.

The present paper deals with the kinetics of nitrogen uptake by 6 marine diatoms isolated from various regions of the Bay of Bengal.

2. Materials and methods

2.1 *Diatoms*

Achnanthes hauckiana Grun., *Amphora coffeaeformis* (Agardh) Kutz., *Fragilaria pinnata* Ehr., *Synedra tabulata* (Agardh) Kutz., *Thalassiosira weissflogii* (Grun.) Fryxell et Hasle (= *Thalassiosira fluviatilis* Hustedt) and *Triceratium dubium* Brightwell were made axenic by antibiotic treatment (Sivasubramanian and Rao 1983) and maintained in Guillard's F/2 medium.

These diatoms were obtained from Madras University Botany Laboratory Culture Collection. The information regarding the collection site and month and year of isolation is given in table 1.

Cultures were grown at 24 ± 1°C in a thermostatically controlled room and illuminated with cool white fluorescent lamps (Sylvania 40 W) at an intensity of 2000 lux in a 12:12 light dark regime. Cultures were continuously aerated with sterile humidified air using an aquarium pump. Nutrient uptake experiments were also conducted under the same conditions in which the cultures were grown. Nitrite was estimated by the diazo-coupling method of Snell and Snell (1949), nitrate as nitrite after reduction by passage through a Cd-Cu column (Strickland and Parsons 1972) and NH_4^+ estimation was by the phenol hypochlorite method (Solorzano 1969).

Table 1. Diatom cultures used in the present study.

Diatoms	Collection site	Isolated by	Month and year of isolation
Achnanthes hauckiana	Pitchavaram	S Deviprasad	March 1977
Amphora coffeaeformis	Karwar	P M Sreelatha	March 1978
Fragilaria pinnata	Madras Harbour	P M Sreelatha	March 1978
Synedra tabulata	Mahabalipuram	P M Sreelatha	August 1977
Thalassiosira weissflogii	Ennore	S K Subramanian	August 1979
Triceratium dubium	Pitchavaram	S Deviprasad	March 1977

To prepare nitrate-depleted cells prior to uptake studies, diatoms were grown in F/2 medium with 25 μM nitrite. Cultures were incubated for 2–3 days till the nitrite in the medium was depleted completely (Eppley *et al* 1969). Cultures were then divided into aliquots and enriched with nitrate, nitrite or ammonium at the beginning of the experiments. Aliquots were incubated for 10–30 min at 24 ± 1°C and 2000 lux light intensity with occasional shaking and at the end of the incubation period, cells were harvested by filtration. Cell-free filtrates were analysed for NO_2^-, NO_3^- or NH_4^+ and uptake was calculated as the difference between the concentration added and that remaining at the end of the experiment. Incubation times were designed to give about 50% uptake of substrate at low substrate concentrations or 2 μmol/litre uptake at higher levels. Uptake of NO_2 and NH_4^+ was linear with time but NO_3^- showed a lag phase. This lag was avoided in these N-depleted cells by preincubation for 3–4 h with 1 μM nitrate (Eppley *et al* 1969). Since some diatoms excreted nitrite during short term uptake the amount of nitrite excreted was also taken into account while carrying out calculations on nitrate uptake.

A linear transformation of the Michaelis-Menten equation: $S = V_{\max}(S/V - K_s)$ was used. Values of $V_{\max}$ and K_s were calculated by linear regression analysis using this equation and the Woolf Plot: $S/V = (1/V_{\max})S + (K_s/V_{\max})$ with S/V as the ordinate and S as the abscissa (as used in Eppley *et al* 1969). In this transform K_s is given as the X-intercept and $V_{\max}$ as the slope of the regression equation of S on (S/V).

3. Results

3.1 V_{max} and K_s

Nitrogen deficient cultures were employed in short term uptake (< 30 min) experiments carried out with different concentrations of KNO_3, $NaNO_2$ and NH_4Cl. Velocity of uptake (v) was plotted against substrate concentration (S). S/V vs S plots were used in preference to other linear transformation as they gave the best spread of the experimental points. Results are given in figures 1–3.

S. tabulata showed the lowest K_s typical of low nutrient species, with most of the points approaching $V_{\max}$, with no points at concentrations lower than K_s. Eppley *et al* (1969) also encountered a similar problem while working with *Skeletonema costatum*. All the other diatoms showed convincing hyperbolas. *F. pinnata* showed a very high K_s while *A. coffeaeformis* and *T. weissflogii* showed intermediate K_s. It was earlier reported (Eppley *et al* 1969) that uptake of nitrogen in some diatoms could be correlated to size of individual diatoms species. To verify this, mean surface area and volume of these diatoms were calculated and are presented along with $V_{\max}$ and K_s values (Table 2). No apparent correlation could be observed between K_s and cell size. *A. hauckiana*, the smallest of the diatoms studied showed a K_s of 3·5 μM for NO_3^- uptake whereas *S. tabulata* which was about 5 times bigger than *A. hauckiana* showed the lowest K_s (0·1 μM). *T. weissflogii* and *T. dubium* showed similar K_s for NO_3^- although the volume of *T. weissflogii* was 10 times lesser than *T. dubium*. *F. pinnata* which was smaller than *S. tabulata* showed the highest K_s.

Similarly, no correlation could be obtained between K_s for NO_2^- and NH_4^+ uptake and cell size of diatoms. *T. dubium*, the largest of the diatoms studied showed a K_s of 9·5 μM for NO_2^- uptake whereas *A. hauckiana* the smallest, showed nearly two times

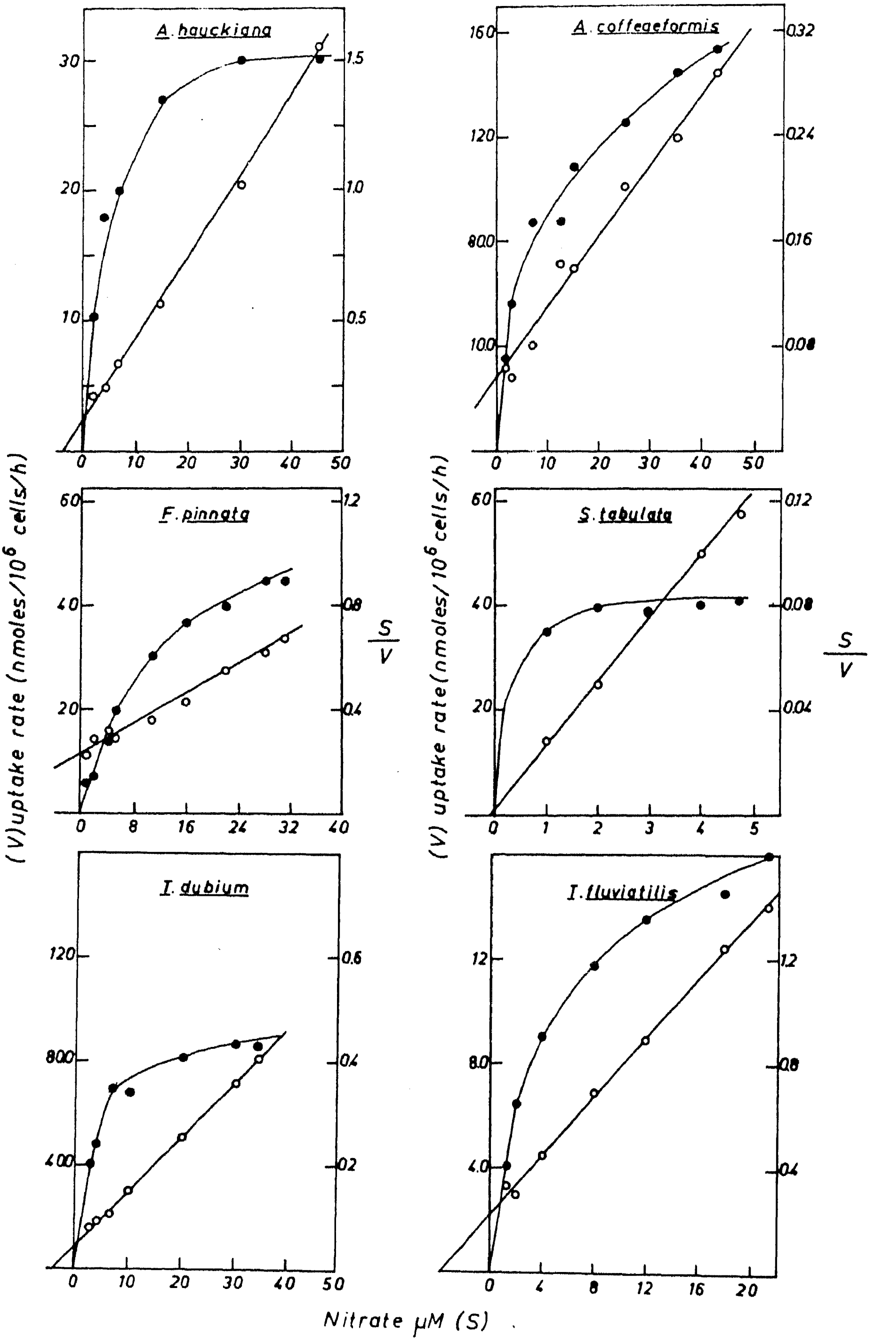

Figure 1. Nitrate uptake rates (v) of different diatoms. Half-saturation constant (K_s) is given as the negative S-intercept of the linear regression of (S/V) vs S.

its K_s. *F. pinnata* and *A. coffeaeformis* with comparable dimensions showed a great difference in K_s. *S. tabulata* showed a K_s, 48 times lower than that of *T. weissflogii* although it was twice larger.

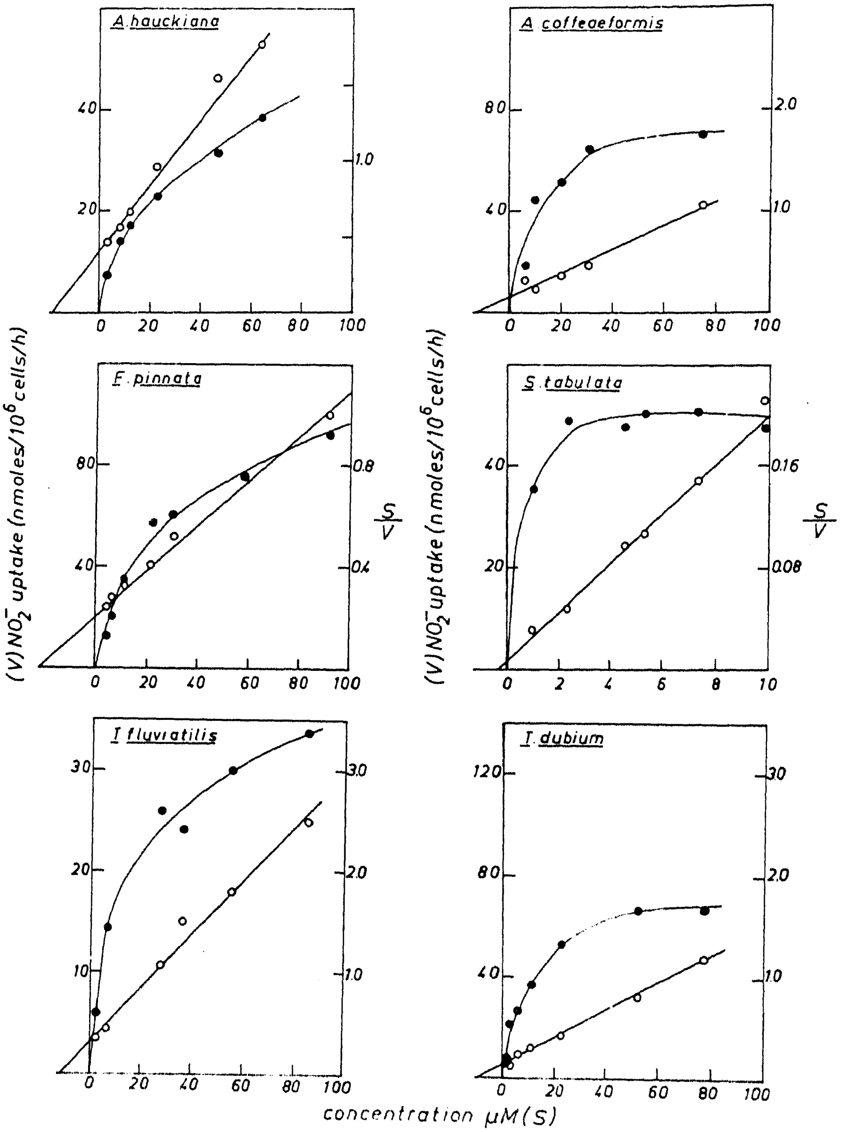

Figure 2. Nitrite uptake rates (v) of different diatoms. Half saturation constant (K_s) is given as the negative S-intercept of the linear regression of (S/V) vs S.

A. hauckiana, *F. pinnata* and *T. weissflogii* which were smaller than *S. tabulata* showed K_s for NH_4^+ uptake greater than 21 μM. Unlike for NO_3^- and NO_2^- uptake, *T. dubium* the largest diatom showed a very high K_s for NH_4^+ uptake.

3.2 *Light and dark uptake of NO_3^-, NO_2^- and NH_4^+*

Uptake experiments were carried out in dark and in different light intensities ranging from 0·5–10 Klux. The velocities of uptake were calculated and the results are given

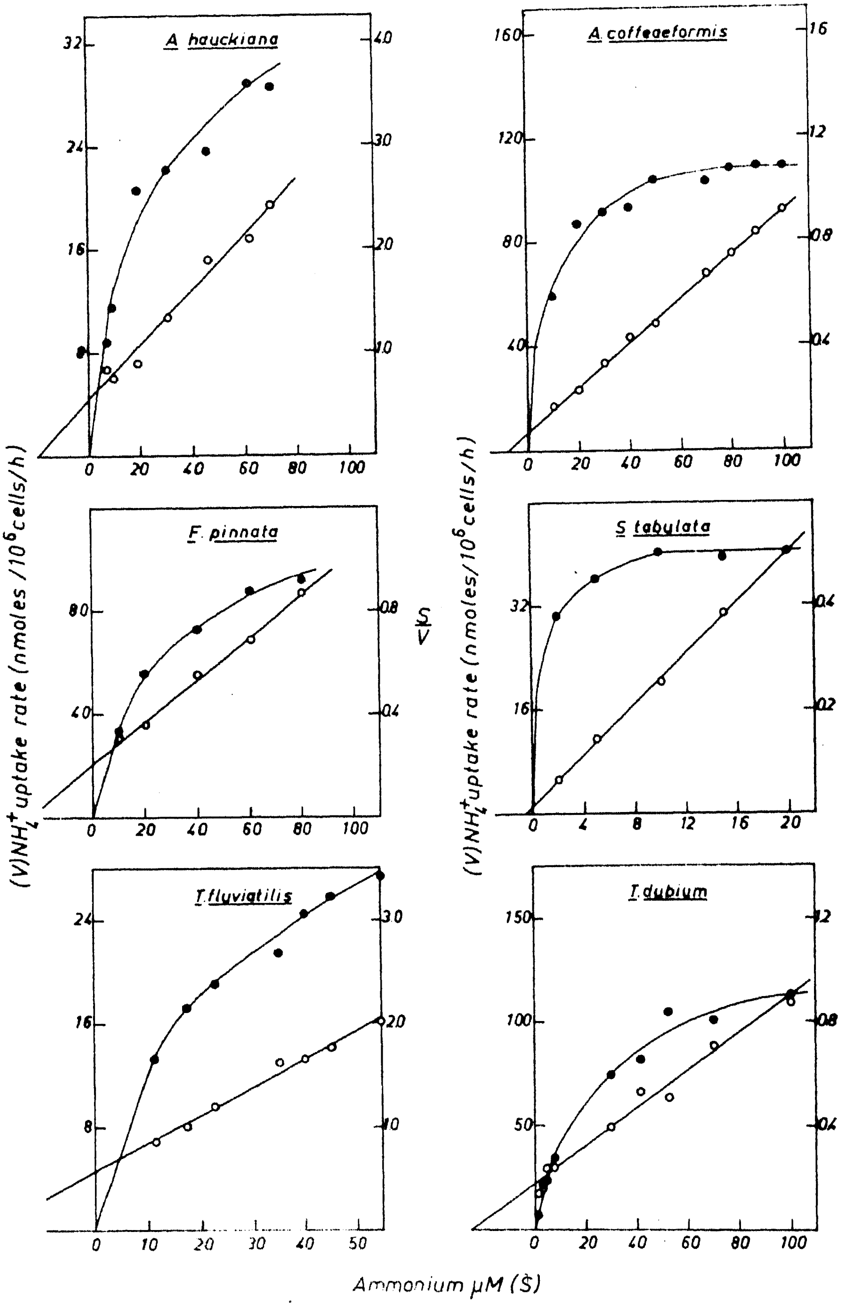

Figure 3. Ammonium uptake rates (v) of different diatoms. Half saturation constant (K_s) is given as the negative S-intercept of the linear regression of (S/V) vs S.

in table 3. All the diatoms were able to take up nitrate in dark, the highest being recorded in *F. pinnata* which was about 43% of the uptake at saturating light intensity. There was no dark uptake of NO_2^- in *A. hauckiana* and *T. weissflogii*. Dark uptake of NO_2^- was observed in other diatoms. In *A. coffeaeformis*, the dark uptake

Table 2. V_{max} and K_s for uptake of NO_3^-, NO_2^- and NH_4^+ by different diatoms.

Diatoms	Cell density[a] ($\times 10^4$ ml)	Mean surface area (μm^2)	Average volume (μm^{-3})	NO_3^- V_{max}nmol 10^6 cells h	NO_3^- K_s (μm)	NO_2^- V_{max}nmol 10^6 cells h	NO_2^- K_s (μm)	NH_4^+ V_{max}nmol 10^6 cells h	NH_4^+ K_s (μm)
Achnanthes hauckiana	40	171·6	220·4	30·5	3·5	43·0	19·5	37·4	21·0
Amphora coffeaeformis	80	626·1	1503·4	177·5	10·5	81·5	12·0	108·0	7·0
Fragilaria pinnata	80	465·0	787·5	69·5	15·0	119·0	27·0	123·0	25·0
Synedra tabulata	35	495·3	991·9	42·0	0·1	50·0	0·3	40·0	0·5
Thalassiosira weissflogii	60	375·7	548·0	18·0	4·0	36·5	12·0	36·7	21·3
Triceratium dubium	40	2816·2	5760·0	96·0	4·0	66·0	9·5	135·0	24·5

[a] For uptake measurements the following diatom cell densities were maintained throughout the investigation.

[b] V_{max} represents maximum velocity reached when uptake experiments were performed at different substrate concentrations under a fixed light intensity of 2000 lux.

Table 3. Uptake of NO_3^-, NO_2^- and NH_4^+ in light and dark

Diatoms	NO_3^- uptake			NO_2^- uptake			NH_4^+ uptake		
	V^*_{max}	Velocity of dark uptake	Dark uptake as % of V_{max}	V^*_{max}	Velocity of dark uptake	Dark uptake as % of V_{max}	V^*_{max}	Velocity of dark uptake	Dark uptake as % of V_{max}
Achnanthes hauckiana	37·0	15·0	40·5	23·0	0·0		24·0	8·0	33·3
Amphora coffeaeformis	100·0	35·0	35·0	131·0	18·0	13·7	94·0	20·0	21·3
Fragilaria pinnata	52·0	22·5	43·3	80·0	14·0	17·5	80·0	28·0	35·0
Synedra tabulata	25·0	5·0	20·0	9·0	5·0	55·6	47·0	20·0	42·5
Thalassiosira weissflogii	18·0	6·0	33·0	11·0	0·0		23·0	4·5	19·6
Triceratium dubium	95·0	30·0	31·6	42·0	7·0	16·6	76·0	16·0	21·0

*V_{max} in mol 10^9 cells h^{-1} represents the maximum velocity reached when uptake experiments were performed at different light intensities with an initial substrate concentration of 20 μM

rate was about 14% of V_{max} while in *S. tabulata* it was 35%. All the diatoms were able to take up NH_4^+ in dark. Dark uptake as percentage of V_{max} ranged from 20–43% (table 3).

3.3 *Nitrate uptake and nitrite release by diatoms*

Actively grown cultures were inoculated into N-free F/2 medium amended with 0·52 mM NO_3^- and incubated for 15 days. Periodic observations were made on growth by taking cell counts on alternate days. Disappearance of nitrate from and release of nitrite into the medium were followed during the period of observation (figure 4). *F. pinnata*, *T. dubium* and *A. coffeaeformis* depleted more than 75% of the nitrate present in the medium within 5 days. *S. tabulata* showed very slow uptake of nitrate. All diatoms excreted nitrite into the medium and *A. hauckiana* excreted more nitrite than the others. Nitrite was observed in the medium upto 10 days after which it was reutilized.

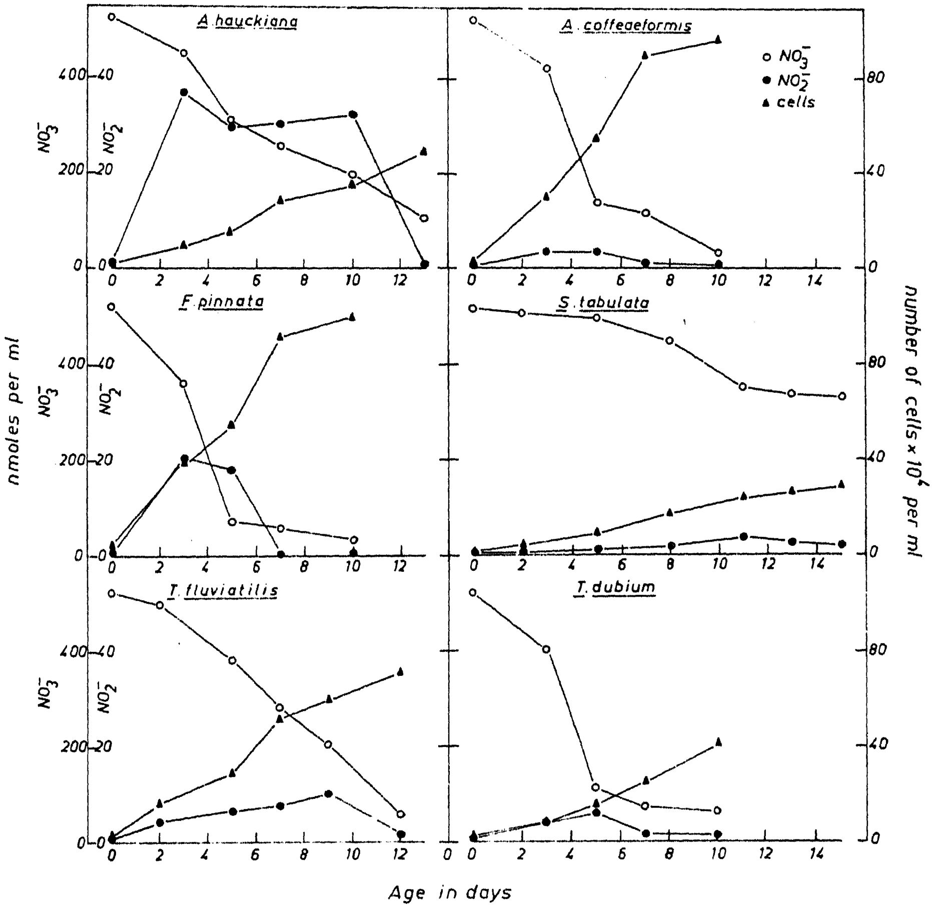

Figure 4. Growth, nitrate uptake and nitrite release by different diatoms.

3.4 *Effect of nitrite on nitrate uptake*

Preliminary studies indicated that in all diatoms uptake rates were faster when only NO_3^- or NO_2^- was supplied separately but the rates were inhibited when both were present together. Nitrate uptake studies were carried out in the presence of different concentrations of nitrite ranging from 2·5–20 μM NO_2^-. Values of K_s for KNO_3^- uptake by these diatoms are given in table 4. *S. tabulata* could not be employed in these studies due to its very low K_s, the rate of uptake being completely inhibited even with the lowest concentration of competitor used for the other diatoms.

3.5 *Effect of NH_4^+ on NO_3^-, NO_3^- on NO_2^-, NH_4^+ on NO_2^-, NO_3^- on NH_4^+ and NO_2^- on NH_4^+ uptake*

NO_3^- uptake experiments were carried out in the absence of and in the presence of NH_4^+ in different concentrations ranging from 5–40 μM. S vs V graphs and Lineweaver-Burk plots were obtained for different diatoms. From these, K_s and V_{max} were calculated and are given in table 5. The result showed a non-competitive type of inhibition. S vs V graphs and Lineweaver-Burk plots were obtained for NO_2^- uptake in the presence of different concentrations of NO_3^-. The inhibitory effect of NO_3^- was greater than the reverse situation (table 6). Nitrite uptake studies were carried out in the presence of different concentrations of NH_4^+ ranging from 5–40 μM. The inhibitory effect of NH_4^+ on NO_2^- uptake was non-competitive, with changes in V_{max} and no significant changes in K_s (table 7). V_{max} and K_s for NH_4^+ uptake were not significantly affected by the presence of NO_3^- (table 8). On the contrary NH_4^+ had a marked influence on the uptake of NO_3^- as discussed earlier. The presence of NO_2^- did not affect the uptake rates of NH_4^+ in all the diatoms. V_{max} and K_s did not change indicating that NO_2^- had no effect on NH_4^+ uptake.

3.6 *Effect of metabolic inhibitors on nitrogen uptake*

Short term uptake experiments were carried out in dark and light in the presence of potassium cyanide (KCN), *p*-chloromercuribenzoate (PCMB) and 3-(3,4-dichlorophenyl) 1, 1-dimethylurea (DCMU) with 10 μM NO_3^-, NO_2^- or NH_4^+ as initial concentration. The results are given in tables 8–10. The results given in table 9 show that PCMB inhibited light and dark NO_3^- uptake rates by 75–86·2%. KCN was less effective. DCMU inhibited light uptake rates by 24–84·8%. Since PCMB is known to

Table 4. K_s for NO_3^- uptake in the presence of NO_2^-.

	NO_2^- (μM)				
Diatoms	0·0	2·5	5·0	10·0	20·0
Achnanthes hauckiana	3·6	5·0	6·7	9·1	11·8
Amphora coffeaeformis	10·5	20·0	22·2	33·3	40·0
Fragilaria pinnata	14·3	21·0	33·3	40·0	40·0
Thalassiosira weissflogii	4·0	7·4	10·5	18·2	22·2
Triceratium dubium	4·0	6·9	8·7	10·5	11·8

Table 5. Short term (< 30 min) NO_3 uptake in the presence of NH_4^+.

Diatoms	NH_4^+ (μM)	V_{max}^a	K_s (μM)
Amphora coffeaeformis	0·0	181·0	10·0
	5·0	48·80	10·0
	10·0	40·80	10·8
	20·0	38·50	12·1
	40·0	34·50	12·5
Achnanthes hauckiana	0·0	33·30	3·9
	5·0	8·0	4·0
	10·0	6·25	4·2
	20·0	5·40	4·3
	40·0	4·80	4·3
Fragilaria pinnata	0·0	82·0	15·0
	5·0	45·50	15·0
	10·0	41·70	20·0
	20·0	33·30	21·0
	40·0	31·30	22·0
Thalassiosira weissflogii	0·0	18·20	4·0
	5·0	6·70	4·0
	10·0	5·47	4·2
	20·0	4·80	4·3
	40·0	4·25	4·4
Triceratium dubium	0·0	111·0	4·0
	5·0	44·0	4·0
	10·0	32·30	4·4
	20·0	27·0	4·5
	40·0	25·0	5·0

[a] n mol/10^6 cells/h.

Table 6. K_s for NO_2 uptake in the presence of NO_3.

	K_s (μM)				
	NO_3 concentration (μM)				
Diatoms	0·0	2·5	5·0	10·0	20·0
Achnanthes hauckiana	20·0	33·3	66·7	68·0	100·0
Amphora coffeaeformis	12·5	20·0	33·3	50·0	80·0
Fragilaria pinnata	26·6	44·4	66·7	100·0	101·0
Thalassiosira weissflogii	12·5	25·0	36·4	50·0	66·7
Triceratium dubium	9·5	25·0	40·0	50·0	66·7

interfere with the catalytic activity of proteins by binding to sulfhydryl groups, it is very likely that the major part of NO_3^- uptake in these diatoms was by carrier mediated active uptake with a very small contribution by passive diffusion, as none of the metabolic inhibitors completely inhibited NO_3^- uptake. In *S. tabulata*, DCMU- and PCMB-inhibited light uptakes were roughly the same showing that the active uptake of NO_3^-, was mediated by energy derived mostly from photosynthesis.

Results given in table 10 show that KCN inhibited NO_2^- uptake completely. PCMB also inhibited NO_2^- uptake significantly. In *A. hauckiana* and *T. weissflogii*,

Table 7. Short term (< 30 min) NO_2^- uptake in the presence of NH_4^+.

Diatoms	NH_4^+ (μM)	V^a_{max}	K_s (μM)
Achnanthes hauckiana	0·0	44·4	20·0
	5·0	18·2	22·2
	10·0	14·8	22·2
	20·0	11·8	23·5
	40·0	11·1	24·0
Amphora coffeaeformis	0·0	76·9	12·5
	5·0	34·5	13·3
	10·0	25·0	14·8
	20·0	20·8	16·3
	40·0	18·9	16·3
Fragilaria pinnata	0·0	120·0	27·1
	5·0	40·1	33·3
	10·0	22·2	36·3
	20·0	16·7	37·0
	40·0	11·8	37·0
Thalassiosira weissflogii	0·0	33·3	12·5
	5·0	16·7	13·1
	10·0	11·1	13·1
	20·0	8·7	13·0
	40·0	6·1	13·0
Triceratium dubium	0·0	70·0	10·0
	5·0	35·7	11·2
	10·0	25·0	11·3
	20·0	16·7	11·3
	40·0	12·5	11·3

[a] n mol/10^6 cells/h.

Table 8. Short term (< 30 min) NH_4^+ uptake in the presence of NO_3^-.

Diatoms	NO_3^- (μM)	K_s (μM)	V^a_{max}
Achnanthes hauckiana	0	21·2	80·0
	5–40	25·0	72·7
Amphora coffeaeformis	0	7·1	116·0
	5–40	7·0	113·0
Fragilaria pinnata	0	25·0	117·0
	5–40	26·6	117·0
Thalassiosira weissflogii	0	21·0	40·0
	5–40	23·5	40·0
Triceratium dubium	0	25·0	148·0
	5·40	25·0	142·9

[a] n moles/10^6 cells/h.

DCMU inhibited NO_2^- uptake completely showing that photosynthetic energy alone was utilized for active NO_2^- uptake. In other diatoms, DCMU inhibited the light uptake of NO_2^- by 68–85% thereby indicating that uptake was mediated mainly by photosynthetically derived energy and to a lesser extent by respiratory energy.

Table 9. Effect of metabolic inhibitors on NO$_3^-$ uptake by different diatoms. Nitrate uptake (v) rates (n mol/10^6 cells/h).

			Inhibitors				
	Control		KCN (1·0 mM)		PCMB (50 μM)		DCMU (2·5 μM)
Diatoms	Light	Dark	Light	Dark	Light	Dark	Light
Achnanthes hauckiana	20·0	11·2	18·5 (7·5)	10·4 (7·15)	3·8 (81·0)	2·0 (82·15)	15·2 (24)
Amphora coffeaeformis	92·5	46·2	90·5 (2·1)	38·2 (17·3)	12·8 (86·2)	8·3 (82·0)	42·2 (54·4)
Fragilaria pinnata	35·2	16·8	31·1 (11·6)	12·3 (26·7)	6·2 (82·4)	3·2 (80·95)	18·2 (48·3)
Synedra tabulata	38·2	5·2	30·1 (21·2)	3·2 (38·5)	5·1 (86·6)	1·1 (78·8)	5·8 (84·8)
Thalassiosira weissflogii	13·2	3·15	8·2 (37·9)	2·8 (11·1)	1·6 (87·9)	0·75 (76·2)	3·9 (70·5)
Triceratium dubium	72·1	40·0	71·1 (1·4)	32·0 (20·0)	15·1 (79·1)	8·1 (79·8)	48·1 (33·3)

Values in parantheses denote per cent inhibition over control.

Table 10. Effect of metabolic inhibitors on NO$_2^-$ uptake by different diatoms. Nitrite uptake (v) rates (n mol/10^6 cells/h).

			Inhibitors				
	Control		KCN 1·0 mM		PCMB (50 μM)		DCMU (2·5 μM)
Diatoms	Light	Dark	Light	Dark	Light	Dark	Light
Achnanthes hauckiana	15·0	0·0	0·0 (100)	0·0 (100)	1·5 (90·0)	0·0 (100)	0·0 (100)
Amphora coffeaeformis	40·0	8·5	0·0 (100)	0·0 (100)	5·2 (87·0)	1·23 (85·5)	8·1 (79·8)
Fragilaria pinnata	50·0	12·5	0·0 (100)	0·0 (100)	13·0 (74·0)	8·75 (30·0)	11·35 (77·3)
Synedra tabulata	42·5	15·2	0·0 (100)	0·0 (100)	18·5 (54·5)	12·2 (19·7)	13·75 (68·0)
Thalassiosira weissflogii	15·1	0·0	0·0 (100)	0·0 (100)	0·75 (95·0)	0·0 (100)	0·0 (100)
Triceratium dubium	32·7	5·25	0·0 (100)	0·0 (100)	4·21 (87·1)	3·12 (40·6)	4·75 (85·5)

Values in parantheses denote per cent inhibition over control.

Results presented in table 11 indicated that none of the inhibitors inhibited NH$_4^+$ uptake completely.

4. Discussion

Studies on nutrient requirement of marine and fresh water phytoplankton species show that competition for limiting nutrients is an important factor controlling species abundance and their succession (Eppley *et al* 1969; Titman 1976; Kilham and

Table 11. Effect of inhibitors on NH_4^+ uptake by different diatoms. Ammonium uptake (v) rates (n mol/10^6 cells/h).

Diatoms	Control		Inhibitors				
			KCN (1 μM)		PCMB (50 μM)		DCMU (2·5 μM)
	Light	Dark	Light	Dark	Light	Dark	Light
Achnanthes hauckiana	23·4	8·5	10·2 (56·4)	7·2 (15·3)	22·2 (5·13)	8·2 (3·53)	19·5 (16·7)
Amphora coffeaeformis	75·2	19·5	40·5 (46·1)	12·8 (34·2)	75·5 (−0·39)	18·2 (6·7)	68·25 (9·2)
Fragilaria pinnata	60·0	27·5	38·2 (36·3)	20·5 (25·5)	56·8 (5·3)	26·3 (4·4)	46·3 (22·8)
Thalassiosira weissflogii	19·5	7·72	12·5 (35·8)	6·2 (19·7)	17·7 (9·2)	8·1 (−4·9)	12·3 (36·9)
Triceratium dubium	58·2	12·5	35·2 (39·5)	9·5 (24·0)	45·2 (22·3)	11·5 (8·0)	43·3 (25·98)

Values in parantheses denote per cent inhibition over control.

Kilham 1978). This competition is indicated by the observed differences in the nutrient uptake kinetics of the competing species (Tilman 1977; Turpin and Harrison 1979). Species with high affinity (low K_s) for nutrients seem to occupy oligotrophic waters, while species with low affinity (high K_s) live in eutrophic waters (Eppley *et al* 1969).

Nutrient uptake by diatoms, in this study, followed the classical saturation kinetics (cf. Eppley *et al* 1969). For all the diatoms, uptake was light dependent only at low irradiance showing saturation at high irradiance. The diatoms took up NO_3^- and NH_4^+ both in light and dark though the dark uptake rate was lower by 20–43% of the light saturated rate. On the contrary, nitrite uptake by diatoms was different. *A. hauckiana* and *T. weissflogii* did not show dark uptake of NO_2^- while others showed very little capacity for dark uptake. Based on the effect of metabolic inhibitors, it could be concluded that NO_2^- uptake was driven by energy derived mainly from photosynthesis and a part of it coming from respiration in some diatoms, whereas, with respect to NO_3^- and NH_4^+ uptake 3 possible mechanisms could be observed: (i) Active uptake deriving energy from photosynthesis, (ii) active uptake deriving energy from cellular respiration and (iii) passive diffusion. Since none of the metabolic inhibitors tested could completely inhibit NO_3^- and NH_4^+ uptake, it was concluded that their uptake could also be through passive diffusion. Earlier, Serra *et al* (1978) reported that in the diatom *Skeletonema costatum* both simple diffusion and carrier-mediated transport were operative during NO_3^- uptake. Eppley *et al* (1969) reported that organisms with high K_s for NO_3^- uptake usually had high K_s for NH_4^+ uptake as well. A similar trend was noticed in these diatoms in the present study.

Eppley *et al* (1969) studied the kinetics of NO_3^- and NH_4^+ uptake by 16 species of marine phytoplankton and reported that K_s varied approximately in proportion to cell size and inversely with specific growth rate. In the present work no such correlation could be obtained between cell size and K_s for nutrient uptake. An inverse relationship between the specific growth rate and the K_s as reported by them was also not obtained here. *S. tabulata* isolated from a nutrient-poor habitat showed

Table 12. Half-saturation constants for uptake of NO_3^-, NO_2^- and NH_4^+ by various diatoms.

Organisms	Reference	NO_3^- K_s (μM)	NO_2^- K_s (μM)	NH_4^+ K_s (μM)
Chaetoceros gracilis	Eppley *et al* (1969)	0·1		0·2
Cyclotella nana Cl-3H	Carpenter and Guillard (1971)	1·87		…
Cyclotella nana 13 1	Eppley *et al* (1969)	0·3, 0·7		0·4
Thalassiosira pseudonana	Eppley and Renger (1974)	0·75	…	0·4
Skeletonema costatum	Eppley *et al* (1969)	0·5, 0·4		3·6, 0·8
Biddulphia aurita	Underhill (1977)	0·91–3·19		…
Ditylum brightwellii	Eppley and Coatsworth (1968)	[illegible]	2·1	1·1
Pleurosigma aestuarii	Rao and Sridharan (1980)	2·4	1·7	5·6
Achnanthes hauckiana	Present study	3·5	19·5	21·0
Amphora coffeaeformis	Present study	10·5	12·0	7·0
Fragilaria pinnata	Present study	15·0	27·0	25·0
Synedra tabulata	Present study	0·1	0·25	0·5
Thalassiosira weissflogii	Present study	4·0	12·0	21·25
Triceratium dubium	Present study	4·0	9·5	24·5

very low K_s when compared to other diatoms derived from localities rich in nutrients which showed fairly high K_s. Table 12 shows a comparison of K_s values obtained in the present study with those published by earlier workers.

Laboratory cultures, though grown in high nutrient culture medium for many years, retain their characteristic uptake curves (Carpenter and Guillard 1971; Underhill 1977). The maintenance medium for diatom cultures has no relevance to the nutrient status of the habitat from which initial isolations are made. Therefore, the fact that half-saturation constants for N-uptake by species are not in anyway influenced by the culture medium is very significant as the observed rate will then truely be indicative of the N-status of the habitat (Rao and Sridharan 1980). In the present work, the diatoms showed different K_s values for nitrogen uptake in spite of the fact that they were grown in F/2 medium since isolation. There was good correlation between the observed K_s and nutrient status of the habitats. Excepting *S. tabulata*, all the other diatoms showed very high K_s values for nitrogen uptake. Since these diatoms were isolated from waters rich in nutrients (table 1) the low substrate affinities exhibited by these diatoms may be an adaptive mechanism against eutrophication.

Studies on the interaction between NO_3^-, NO_2^- and NH_4^+ revealed that NH_4^+ inhibited both NO_2^- and NO_3^- uptake non-competitively whereas NO_3^- and NO_2^- inhibited each other in a competitive manner (cf. Eppley and Coatsworth 1968). Bilbo *et al* (1981) working with *Phaeodactylum tricornutum* concluded that the inhibition by nitrate of nitrite uptake is of competitive type. But Cresswell and Syrett (1981, 1982) working with the same diatom were unable to confirm this conclusion. They have shown that when ammonium, nitrate and nitrite are present together, the diatom takes up NH_4^+ first and then NO_3^-, NO_2^- being absorbed only when the other two ions have completely disappeared from the medium. This might only be an apparent effect since all diatoms used in our study excrete some amount of NO_2^- during NO_3^- uptake. Similar inhibitory effects have been reported in other organisms such as *Codium* (Hanisak and Harlin 1978), *Laminaria* (Harlin and Craigie 1978) and *Ditylum* (Eppley and Coatsworth 1968). Nitrite inhibition of nitrate uptake does not occur in *Codium, Ditylum* or *Skeletonema* (Serra *et al* 1978). Olson *et al* (1980) have reported a competitive inhibition between NO_3^- and NO_2^- in *Thalassiosira pseudonana.* Similarly, we have also observed a clear cut competition between NO_2^- and NO_3^- for active sites in all the diatoms studied. The NO_3^- inhibition of NO_2^- uptake was greater than the reverse situation. Studies with metabolic inhibitors have shown that NO_3^- is taken up actively (supported by both photosynthesis and respiration) and by a passive diffusion whereas NO_2^- is taken up only through photosynthesis-mediated active uptake. So, when both NO_3^- and NO_2^- are present together, NO_2^- could competitively inhibit only the active uptake of NO_3^- and not its passive diffusion. This could account for the slightly higher uptake of NO_3^- in the presence of NO_2^-. Terry (1982) working with *Phaeodactylum tricornutum* confirms that uptake rate for NO_3^- is reduced by addition of NH_4^+ to the medium. In *Skeletonema costatum,* Dortch and Conway (1984) have demonstrated that simultaneous nitrate and ammonium utilization is more frequent and also added that ammonium-inhibition of nitrate-uptake is not only controlled by internal ammonium and total amino acids as previously believed, but also depends on both the preconditioning of the cells, whether cultivated on nitrate and/or ammonium and the respective concentration of the two compounds in the medium at the time of observation. In our study the cultures used for NH_4^+ uptake experiments were grown in nitrite. It has been suggested that such an inhibition may be at the level of nitrate reductase (NR) (Syrett and Morris 1963; Ferguson 1969). In the presence of NH_4^+ there is immediate inhibition of NO_3^- uptake (Ohmori *et al* 1977; Pistorious *et al* 1978; Flores *et al* 1980). For some algae this inhibition is total in the presence of 0·5–1 μM NH_4^+ concentration (Morris and Syrett 1963; Eppley *et al* 1969; McCarthy and Eppley 1972; McCarthy *et al* 1977). Inhibition is reversible and the ability of cells to utilize NO_3^- is restored immediately after exhaustion of NH_4^+ in the external medium. This type of behaviour was observed in *A. coffeaeformis* (Rao V N R and Sivasubramanian V, unpublished results). Cresswell and Syrett (1979) demonstrated that in *Phaedactylum tricornutum* NO_3^- uptake was inhibited at the transport step. Flores *et al* (1980) observed that in the presence of specific inhibitors of NH_4^+ assimilation (methionine, sulphoximine and azaserine) NH_4^+ did not inhibit NO_3^- uptake by blue-green algae suggesting that involvement of NH_4^+ assimilation was essential for control of NO_3^- utilization (see also Guerrero *et al* 1981). In the present study it was observed that even cultures grown with 100 μM tungstate (an inhibitor of NR activity) could take up NO_3^- at the same rate as that of untreated cultures, thus ruling out the possibility of NH_4^+, controlling NO_3^- uptake through NR (Rao V N R and Sivasubramanian V, unpublished results).

Acknowledgements

The authors wish to thank the Director for his keen interest and for providing the facilities and one of the authors (VS) thanks the University Grants Commission, New Delhi for a fellowship.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 4, April 1988, pp. 89–98.
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Uptake and assimilation of nitrogen by marine diatoms—II. Kinetics of nitrogen assimilation

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MS received 26 September 1987; revised 12 March 1988

Abstract. A comparison of K_M values for nitrate and nitrite reductases with uptake capacities of 6 marine diatoms indicated that intracellular accumulation of NO_3^- and NO_2^- is necessary for efficient functioning of the enzymes. *Amphora coffeaeformis*, *Thalassiosira weissflogii* and *Synedra tabulata* accumulated very little NO_2^- whereas *Triceratium dubium*, *Achnanthes hauckiana* and *Fragilaria pinnata* accumulated 0·02 to 1·5 mM NO_2^-. Studies with inhibitors of nitrate and nitrite reductase indicated that nitrite reductase could be located in a different compartment such as chromatophores as in higher plants. NH_4^+ was also accumulated by the diatoms. Studies on the kinetics of glutamine synthetase and glutamate dehydrogenase indicated that the primary enzyme involved in NH_4^+ assimilation could be glutamate dehydrogenase, because of its low $K_M^{NH_4^+}$ and relatively greater activity than glutamine synthetase. Intracellular analysis of NH_4^+ revealed that its concentration was well below the $K_M^{NH_4^+}$ for glutamine synthetase.

Keywords. Diatoms; glutamate dehydrogenase; glutamine synthetase; nitrate reductase; nitrite reductase; nitrogen assimilation.

1. Introduction

Phytoplankton enzymes involved in nitrogen assimilation, especially nitrate reductase (NR) and nitrite reductase (NiR) have been extensively used in laboratory and field studies to estimate nitrogen utilization capacity of cells (Eppley *et al* 1969; Eppley and Rogers 1970; Collos and Slawyk 1976; Slawyk and Rodier 1986). These enzyme assays seem especially useful for studying the time course of phytoplankton bloom because it provides a sensitive measure of the initiation and cessation of nitrate assimilation. However, these two enzymes do not account for consumption of other nitrogen compounds such as ammonium, especially in oligotrophic areas (Eppley and Peterson 1979). Activity of the enzyme glutamate dehydrogenase (GDH) has been widely used to estimate rates of utilization of ammonium (Ahmed *et al* 1977; Dortch *et al* 1979). However, doubts have recently been raised regarding the efficient functioning of GDH at *in vitro* NH_4^+ concentrations (Falkowski and Rivkin 1976). There are several reports recently, on the probable involvement of glutamine synthetase (GS) as a key enzyme involved in the entry of ammonium into amino acids of marine unicellular algae (Falkowski and Rivkin 1976; Turpin and Harrison 1978; Bressler and Ahmed 1984; Thomas *et al* 1984; Slawyk and Rodier 1986).

In the present study an attempt has been made to compare the affinity of assimilating enzymes (nitrate reductase and nitrite reductase) to their respective substrates in different diatoms with the ability for uptake and accumulation of nitrogenous nutrients from the surrounding environment. In view of the potential importance of GS and GDH for giving information about internal metabolic pathways and rates of overall nitrogen assimilation, we have also made a critical assessment of the relative importance of these two pathways in NH_4^+ assimilation.

2. Materials and methods

2.1 *Diatoms and culture conditions*

Axenic cultures of *Achnanthes hauckiana* Grun., *Amphora coffeaeformis* (Agardh) Kutz., *Fragilaria pinnata* Ehr., *Synedra tabulata* (Agardh) Kutz., *Thalassiosira weissflogii* (Grun.) Fryxell et Hasle (= *Thalassiosira fluviatilis* Hustedt) and *Triceratium dubium* Brightwell were used in the present study. Information regarding their source, culture medium and growth conditions has been given elsewhere (Sivasubramanian and Rao 1988).

2.2 *Estimation of NO_3^-, NO_2^- and NH_4^+*

Nitrite was estimated by the diazo coupling method of Snell and Snell (1949). Nitrate was estimated as nitrite after reduction by passage through a Cd-Cu column (Strickland and Parsons 1972). Ammonia was estimated by the phenol hypochlorite method (Solorzano 1969). Analysis of intracellular NO_3^-, NO_2^- and NH_4^+ was carried out after sonicating the diatom pellets with 2 ml of 5% trichloro acetic acid and neutralizing the supernatant with 0·5 ml of 2 N NaOH.

2.3 *Extraction and assay of NR and NiR*

A wet pellet of 1 g (fresh weight) of diatom material was harvested and washed twice with 0·2 M phosphate buffer (pH 7·9). It was resuspended in 5 ml of 0·2 M phosphate buffer (pH 7·9) containing 1 M dithiothreitol and homogenized in a MSE-150 ultrasonic disintegrator for 10 min keeping the sample tube in ice during operation. The clear supernatant after centrifugation was used for the assay of enzymes.

The reaction mixture for NR assay included 1 ml of enzyme extract, 20 μmol KNO_3, 0·5 μmol NADH and 50 μmol $MgSO_4$ in a volume of 1·8 ml (Eppley *et al* 1969). The activity was stopped by adding 5 ml of cold 90% ethanol and 0·2 ml of 1 M zinc acetate after 30 min incubation. This was centrifuged and the supernatant was analysed for NO_2^- formed.

The reaction mixture of NiR assay (Eppley and Rogers 1970) included 1 ml of enzyme extract; 1·7 ml of phosphate buffer, 0·2 M, pH 7·9; 0·1 ml of methyl viologen solution (40 mg 10^{-1} ml glass distilled water), 0·1 ml of $NaNO_2$ solution (9 mM in water) and 0·1 ml of sodium dithionite solution (60 mg sodium dithionite and 60 mg sodium bicarbonate in 2·5 ml glass distilled water, freshly prepared). Reaction tubes were well stoppered and incubations were carried out at 30°C for 30 min. The reaction was stopped with 0·1 ml of 1 M zinc acetate and 1·9 ml of 95% ethanol. The clear supernatant after centrifugation was taken for NO_2^- analysis.

2.4 *Extraction and assay of ammonia assimilating enzymes*

A diatom pellet of 1 g fresh weight was harvested and washed twice in 0·1 mM imidazole-HCl buffer, pH 7·5, containing 10 mM EDTA and 1 mM dithiothreitol (DTT) and then resuspended in the same buffer containing 1 mM DTT. The diatom suspension was sonicated and clear supernatant was used for the assay of enzymes. GS was assayed by the γ-glutamyl transferase assay of Shapiro and Stadtman (1970). The reaction mixture included in 1 ml, imidazole-HCl buffer 0·04 M, pH 7·5, glutamine 30 mM, $MnCl_2 4H_2O$ 0·3 mM, ADP, trisodium salt, 0·4 mM, sodium arsenate, 20 mM and hydroxylammonium-HCl 60 mM. Incubations were carried out at 37°C for 15 min. The reaction was terminated by adding 2 ml of stop mixture composed of 3·3% ferric chloride, 2% trichloroacetic acid and 0·25 N HCl. The γ-glutamyl hydroxamate was used as standard.

For determination of the K_M for ammonium spectrophotometric assay of inorganic phosphate (Shapiro and Stadtman 1970) as modified by Falkowski and Rivkin (1976) was used. The standard reaction mixture contained: 0·2 ml of 1 M imidazole-HCl, pH 7·5; 0·01 ml of 10 mM NH_4Cl; 0·05 ml of 1 M Na-glutamate, pH 6; 0·05 ml of 60 mM ATP, pH 7; and 0·05 ml of 1·67 M $MgCl_2$. Reaction was initiated by addition of 0·005 ml·enzyme extract. Incubations were carried out at 37°C for 15 min. Reaction was terminated by addition of 1·8 ml ferrous sulphate reagent (0·8% $FeSO_4$, 7 H_2O in 0·015 N H_2SO_4) and 0·15 ml ammonium molybdate reagent (6·6% $(NH_4)_6Mo_7O_{24}4H_2O$ in 7·5 N H_2SO_4). Colour developed was read at 850 nm after one min.

For determination of K_M for glutamate the following assay procedure was followed (Rowe *et al* 1970). One ml of enzyme extract was added to 0·9 ml of assay mixture containing imidazole—HCl buffer, pH 7·5, 50 μmol; $MgCl_2$ 20 μmol; 2-mercaptoethanol 25 μmol; sodium L-glutamate, 200–2000 μmol; hydroxylammonium-HCl 200 μmol; NaATP 10 μmol. Incubations were carried out at 37°C for 15 min. Activity was stopped by addition of 1·5 ml stop-mixture containing 10% $FeCl_3$ 4 ml, 24% trichloroacetic acid 1 ml, 6 N HCl 0·5 ml and distilled water 6·5 ml. The γ-glutamyl hydroxamate formed was measured at 540 nm in a spectronic 20.

Glutamate synthase (GOGAT) and GDH were measured by following the decrease in extinction at 340 nm due to the oxidation of NADPH at 25°C in an UNICAM SP 800 B spectrophotometer. The reaction mixture (Neilson and Doudoroff 1973) for GOGAT and GDH in 2·5 ml contained the following: 10 μmol of 2-oxoglutarate pH 7·8; 0·5 μmol of NADPH, pH 7·8; 20 μmol of glutamine, pH 7·8 (for GOGAT) or 400 μmol of NH_4Cl (for GDH); 200 μmol of imidazole-HCl, pH 7·8; and 0·3 ml of enzyme extract. Controls lacking glutamine for GOGAT or NH_4Cl for GDH were included. Specific activity was expressed as μmol NADPH oxidised per min mg protein using the molar extraction coefficient of NADPH (6220 at 340 nm).

3. Results

3.1 V_{max} and K_M for NR and NiR

NR and NiR were assayed with different concentrations of $NaNO_3$ and $NaNO_2$ in the respective reaction mixtures. The assay mixtures were incubated for 30 min. Shorter incubation times were used with low concentrations in order to deplete less than 25% of the substrate. Activity was calculated n mol of NO_2^- formed (NR) or reduced (NiR) $min^{-1} mg^{-1}$ protein. Lineweaver-Burk plots were used to determine K_M values and V_{max}(figures 1 and 2). *S. tabulata* showed the lowest K_M indicating that it can efficiently assimilate NO_2^- and NO_3^- even at very low intracellular concentrations.

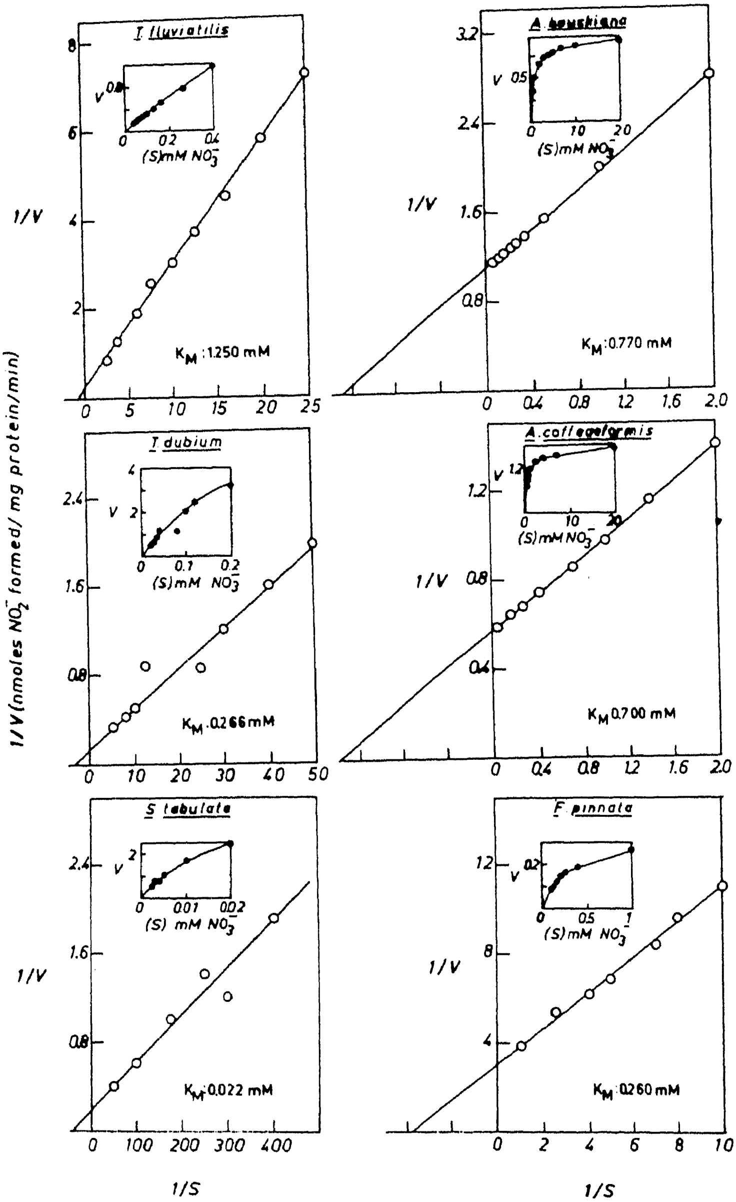

Figure 1. Lineweaver-Burk plots for NADH-NR activity of different diatoms. Inset: NR activity vs substrate (NO_3^-) concentrations. K_M/V_{max} values: *A. hauckiana* 0·77/0·9; *A. coffeaeformis* 0·70/1·8; *F. pinnata* 0·26/0·3; *S. tabulata* 0·02/5·3; *T. fluviatilis* 1·25/4·0; *T. dubium* 0·27/7·7.

3.2 *Effect of feedback inhibitors on NR and NiR in vitro*

NR and NiR were assayed with 50 mM NO_2^- or NO_3^- concentration in the presence of NH_4^+, NO_3^- or glutamate. Results are given in table 1. NH_4^+ did not inhibit NR and NiR *in vitro*. Glutamate had a similar effect on NR and NiR activity. NO_3^- at

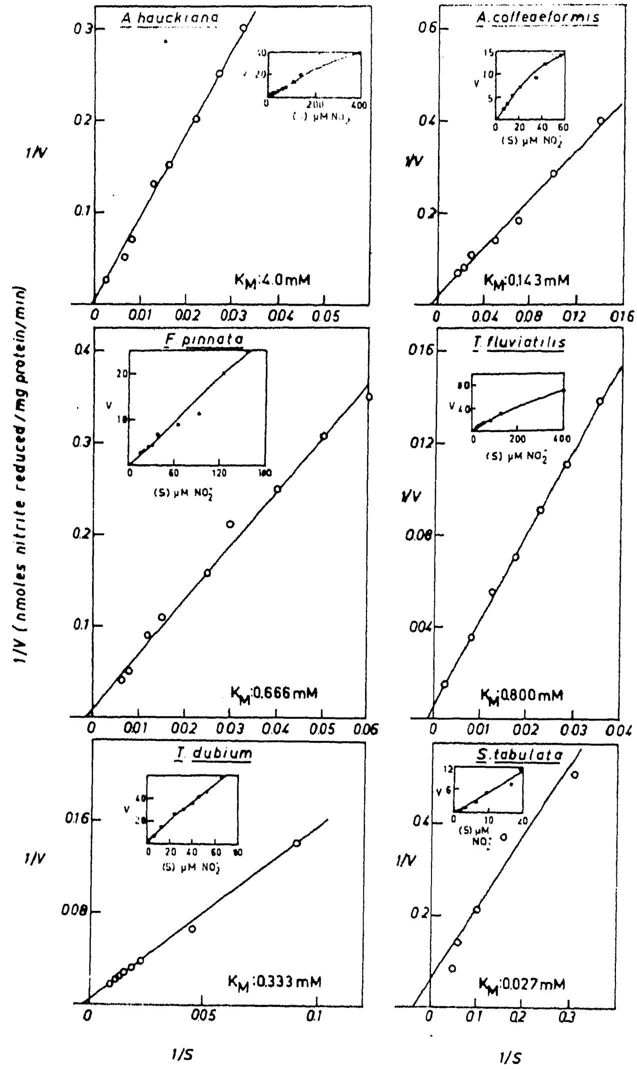

Figure 2. Lineweaver-Burk plots for NiR activity of different diatoms. Inset: NiR activity vs substrate (NO_2^-) concentrations. K_M/V_{max} values: *A. hauckiana* 4·00/200; *A. coffeaeformis* 0·14/50; *F. pinnata* 0·67/135; *S. tabulata* 0·03/17; *T. fluviatilis* 0·80/200; *T. dubium* 0·33/200.

500 μM level inhibited NiR by about 85–100%. NH_4^+ is known to inhibit NO_3^- and NO_2^- uptake in these diatoms (Sivasubramanian and Rao 1988).

Analysis of intracellular NO_3^- in diatoms showed concentrations ranging from 10–50 m mol/l cell volume. Since NO_3^- inhibits NiR *in vitro*, it is possible that NiR could be located in a different compartment as in higher plants.

3.3 V_{max} and K_M for GS and GDH

All the 6 diatoms used in the present study showed GS and GDH activities. The pennate *A. coffeaeformis* and the centric *T. dubium* were selected for a critical

Table 1. Inhibition of NADH-NR and NiR activity *in vitro*.

	NR (n mol of NO_2^- formed/mg protein/min)				NiR (n mol of NO_2^- reduced/mg protein/min)					
		NH_4^+				NH_4^+		NO_3^-		
Diatoms	Control	50 μM	500 μM	Glutamate (500 μM)	Control	50 μM	500 μM	50 μM	500 μM	Glutamate (500 μM)
Achnanthes	0·50	0·48	0·40	0·50	8·5	8·00	8·7	6·2	0·75	8·0
hauckiana		(4·0)	(20·0)	(0·0)		(5·90)	(0·0)	(27·0)	(91·2)	(5·9)
Amphora	0·85	0·80	0·79	0·79	12·8	11·50	12·50	10·9	1·90	11·1
coffeaeformis		(5·9)	(7·06)	(7·06)		(10.16)	(2·34)	(14·8)	(85·2)	(13·3)
Fragilaria	0·25	0·20	0·20	0·24	7·5	6·75	6·80	5·2	0·0	7·0
pinnata		(20·0)	(20·0)	(4·0)		(10·0)	(9·33)	(30·7)	(100)	(6·7)
Synedra	2·80	2·70	2·00	2·70	12·0	11·20	11·70	6·1	1·10	11·20
tabulata		(3·6)	(28·6)	(3·6)		(6·70)	(2·50)	(49·2)	(90·8)	(6·7)
Thalassiosira	0·50	0·48	0·45	0·48	12·5	11·15	11·20	6·2	1·00	11·00
weissflogii		(4·0)	(10·0)	(4·0)		(10·8)	(10·40)	(50·4)	(92·0)	(12·0)
Triceratium	2·50	2·00	2·0	2·28	40·0	39·50	37·50	22·0	3·15	36·2
dubium		(20·0)	(20·0)	(8·8)		(1·25)	(6·25)	(45·0)	(92·1)	(9·5)

Values in parantheses denote per cent inhibition over control.
All values mean of triplicates.

assessment of the relative importance of the two pathways in NH_4^+ assimilation. For determination of K_M^{glu} the biosynthetic assay of GS involving the measurement of γ-glutamyl hydroxamate was used. For determining $K_M^{NH_4^+}$, the assay of inorganic phosphate released in a biosynthetic reaction was followed. K_M values were calculated from linear regression analysis of Lineweaver-Burk plots (figure 3).

GDH was assayed with NADPH. The affinity of GDH to NH_4^+ and α-ketoglutarate ($K_M^{NH_4^+}$ and $K_M^{\alpha\text{-Kg}}$) were determined from linear regression analysis of Lineweaver-Burk plots (figure 4). GOGAT could not be detected in the extracts of *T. dubium* and *A. coffeaeformis*. Table 2 gives a comparison of K_M and V_{max} for GS and GDH. GDH activity in *A coffeaeformis* and *T. dubium* was approximately 3 times more than GS activity at saturating substrate concentrations. GDH had an approximate K_M of 13·2 μM in *A. coffeaeformis* and 182 μM in *T. dubium* for NH_4^+, while the K_M with respect to GS was 1 mM in *A. coffeaeformis* and 0·555 mM in *T. dubium*. In view of the low $K_M^{NH_4^+}$ and relatively high activity of GDH to GS, it is suggested that GDH may be the major assimilatory enzyme involved in the incorporation of NH_4^+ into the amino acid pool in *A. coffeaeformis* and *T. dubium*.

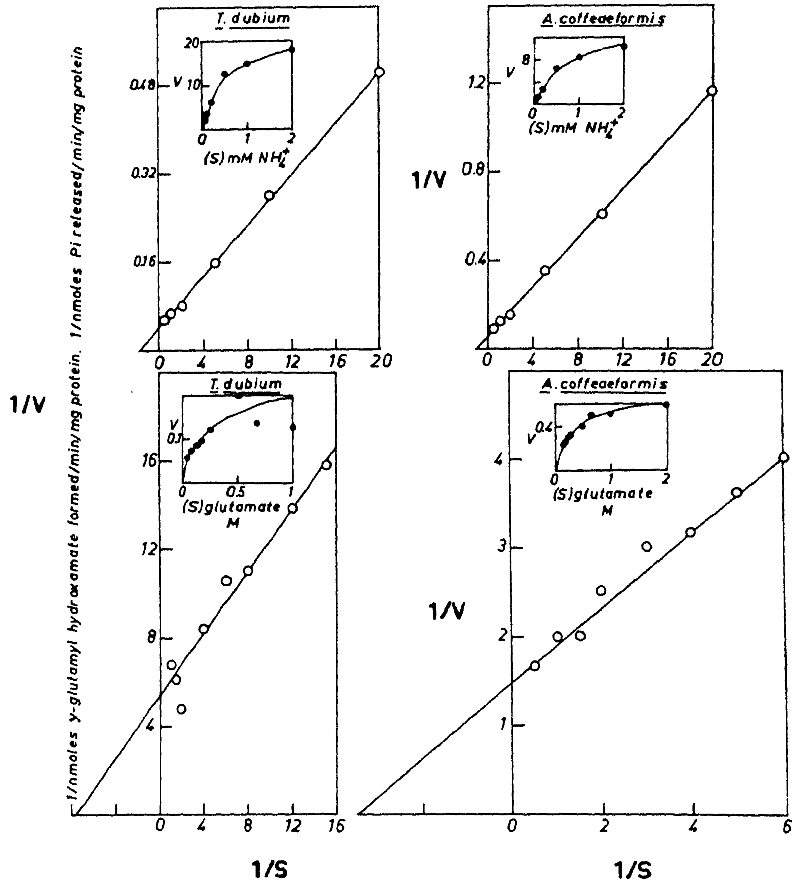

Figure 3. Lineweaver-Burk plots for GS activity in *A. coffeaeformis* and *T. dubium*. Inset: GS activity against substrate concentrations.

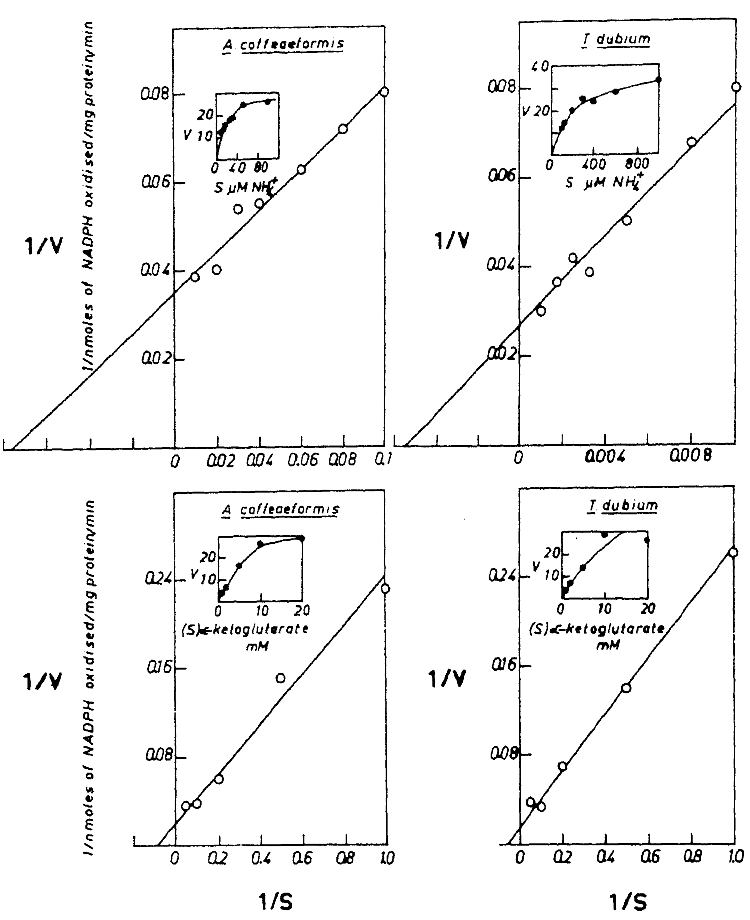

Figure 4. Lineweaver-Burk plots for GDH activity in *A. coffeaeformis* and *T. dubium*. Inset: GDH activity against substrate concentrations.

Table 2. Comparison of K_M and V_{max} for GS and GDH.

Diatoms	Enzyme	$K_M^{NH_4^+}$	$K_M^{\alpha-Kg}$	K_M^{glu}	V^a_{max} n mol/mg protein/min
Amphora coffeaeformis	GS	1·0 mM	—	286 mM	16·6 Pi
	GDH	13·2 μM	11·1 mM	—	47·6 NADPH
Triceratium dubium	GS	0·6 mM	—	132 mM	22·5 Pi
	GDH	182·0 μM	16·6 mM	—	66·7 NADPH

[a] V_{max} at saturating substrate concentrations.
Values mean of triplicates.

The analysis of intracellular NH_4^+ showed 20–50 μmol/l cell volume in *A. coffeaeformis* and 100–200 μmol/l cell volume in *T. dubium* which were well below the $K_M^{NH_4^+}$ for GS.

4. Discussion

K_M values of NR and NiR were 100 times more than the K_S values for uptake (Sivasubramanian and Rao 1988). For efficient functioning of the enzymes, intracellular accumulation of NO_3^- and NO_2^- seemed necessary. Diatoms in the present study accumulated 10–50 mM NO_3^-. Eppley and Rogers (1970) reported that *Ditylum brightwellii* accumulated 40 mM NO_3^-, but less than 10 μM NO_2^-. Analysis of intracellular NO_2^- revealed that *A. coffeaeformis*, *T. weissflogii* and *S. tabulata* accumulated very low amount of NO_2^- ($<10\,\mu$M). *A. hauckiana* and *F. pinnata* accumulated about 0·02–0·05 mM NO_2^- and *T. dubium* accumulated 0·5–1·5 mM NO_2^-. In general the level of NR is higher in organisms grown on NO_3^- and low in organisms grown on NH_4^+ (Guerrero *et al* 1981). Because of this more NO_2^- is usually not accumulated in diatoms. In the present study *A. hauckiana*, *F. pinnata* and *T. dubium* showed higher K_M for NiR than NR. This may partly explain why there was accumulation of NO_2^- in these diatoms. The other diatoms which showed very low accumulation showed higher K_M for NR than NiR. $V_{\max}$ of NiR was also found to be more than that of NR. NO_3^- inhibited NiR *in vitro*. Since there was intracellular accumulation of NO_3^- in these diatoms it is obvious that NiR should be separated from NO_3^- and could be located in a different compartment. Based on chlorophyll fluorescence studies (Kessler and Zumft 1973; Kulandaivelu *et al* 1976) it was suggested that NiR could be located in chloroplasts in green algae. This aspect in diatoms needs further study. Although NH_4^+ repressed the NiR and NR synthesis *in vivo*, it did not inhibit NiR and NR *in vitro*. NH_4^+ was also accumulated in diatoms. The mechanism by which NH_4^+ controls NiR and NR activity *in vivo* is not very clear.

Falkowski and Rivkin (1976) based on their studies in *Skeletonema costatum* proposed that GS is the enzyme primarily responsible for the incorporation of NH_4^+ into the amino acid pool. They were not able to detect GDH with NADPH. Recently evidence indicates that GS coupled with GOGAT is probably the main pathway for the entry of NH_4^+ (Turpin and Harrison 1978; Bressler and Ahmed 1984; Thomas *et al* 1984). Eppley and Rogers (1970) attempted to quantify NH_4^+ assimilation in *Ditylum brightwellii*. They obtained positive results with α-ketoglutarate and NADPH, thus suggesting the presence of GDH. Ahmed *et al* (1977) observed the presence of NAD-GDH activity in 4 of the phytoplankters they studied. Because of the greater activity of GDH than GS and relatively low $K_M^{NH_4^+}$ for GDH than GS it is proposed that GDH may be the primary enzyme responsible for the assimilation of NH_4^+ in *A. coffeaeformis* and *T. dubium*. This may also be true for the other 4 diatoms. GS may become important when nitrogen is available in excess.

Acknowledgments

The authors thank the Director for his keen interest and for providing laboratory facilities. One of the authors (VS) wishes to thank the University Grants Commission, New Delhi for a fellowship.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 2, April 1988, pp. 99–109.
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Diurnal and seasonal variations in the phytoplankton production of lake Kondakarla

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MS received 23 February 1987; revised 30 January 1988

Abstract. Variations in the phytoplankton production of lake Kondakarla were estimated at two levels (surface and at 1 m depth) during a period of 12 months. The gross production values ranged from 0·10–1·40 mg $O_2 l^{-1} h^{-1}$ at surface and from 0·06–0·73 mg $O_2 l^{-1} h^{-1}$ at 1 m depth. Peak values of gross production occurred between 9–12 h or from 12–15 h during the different months at both the levels. The seasonal variation in gross primary production ranged from 2·70 mg $O_2 l^{-1}$ day^{-1} during January 1984 to 9·42 mg $O_2 l^{-1}$ day^{-1} in September 1983 for surface waters and from 1·60 mg $O_2 l^{-1}$ day^{-1} during September 1983 to 5·40 mg $O_2 l^{-1}$ day^{-1} in February 1984 in waters at 1 m depth. The depth integrated production varied from 4·08–13·05 g $O_2 m^{-2}$ day^{-1} while the photosynthetic efficiency ranged from 0·580–4·301%.

Keywords. Phytoplankton production; seasonal and diurnal variation.

1. Introduction

Phytoplankton production of the various water bodies in India has been reported to vary from 0–97·50 g $O_2 m^{-2}$ day^{-1}. The lowest production rates were observed at Ramgarh lake (0·036 g C m^{-2} day^{-1}, Gopal *et al* 1978) followed by Bhimtal and Nainital lakes in Uttar Pradesh (Pande and Singh 1978). Kannan and Job (1980) observed higher production rates at the surface water of Sathiar reservoir except in the months of December and January; and observed that the nutrients are not limiting factors for the gross primary production of this reservoir. Zafar (1986) compared the annual average primary production of the various water bodies around Hyderabad and observed that the values of gross primary production varied from 0·60 g C m^{-3} day^{-1} in Himayatsagar to 5·20 g C m^{-3} day^{-1} in Banjara lake. Venu and Seshavatharam (1984) reported the average primary production at different sites of lake Kondakarla ranged between 1·06–11·66 g C m^{-2} day^{-1}. Ramakrishna (1987) has reported the primary production varied between 0·93–4·96 g C m^{-2} day^{-1} at different locations of lake Kolleru in Andhra Pradesh. Sumitra (1971), Nasar and Datta Munshi (1975), Nasar and Nasar (1978) observed a bimodal fluctuation during the year in the productivity of different fresh water bodies of the country. The photosynthetic efficiency of the inland water bodies of our country was reported to vary from 0·17% in south Indian lakes (Ganapathi 1972; Ganapathi and Sreenivasan 1972; Sreenivasan 1972) to 13·80% in a sewage oxygen pond at Ahmedabad (Jayangoudar *et al* 1970).

Diurnal and seasonal variations in the phytoplankton production and photosynthetic efficiency of the lake waters studied during a period of 12 months from September 1983 to August 1984 are presented here.

2. Study area

Lake Kondakarla is situated between latitudes 17°35′30″ and 17°36′02″ N, longitudes 82°59′27″ and 83°01′0″ E and is about 10 km south of Anakapalle and 50 km south-west of Visakhapatnam city. The lake has an area of about 6·5 km^2 with a catchment area of about 20 km^2. The lake has an inlet, the Munagapaka channel and an outlet to the Bay of Bengal which is about 10 km from the lake (figure 1).

3. Materials and methods

3.1 *Primary production*

The phytoplankton production of the lake waters was estimated following Gaarder

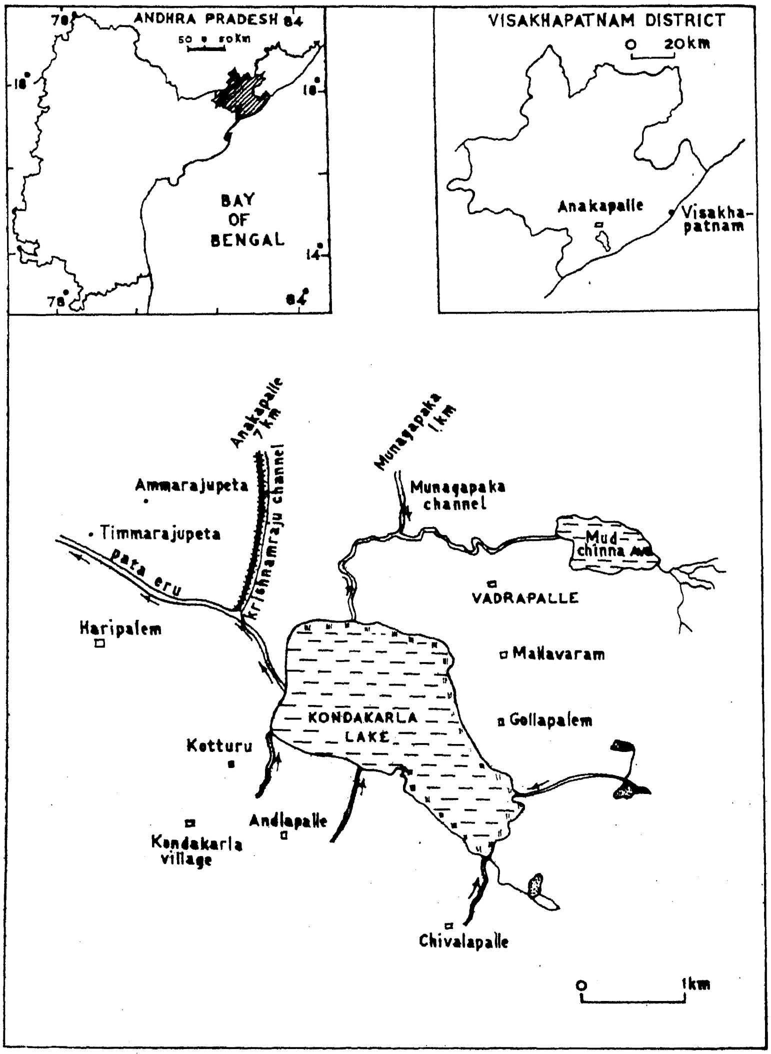

Figure 1. Map of lake Kondakarla.

and Gran's (1927) light and dark bottle technique using 250 cc corning glass bottles. Diurnal variation in the production rate was studied at 3 hourly intervals from dawn to dusk at two levels—at surface and at 1 m depth.

3.2 *Depth integrated production and photosynthetic efficiency*

The total radiation expressed as kcal m^{-2} day^{-1} is computed from the data obtained from the Indian Meteorological Department, Visakhapatnam on the days of the experiment during the study period. The photosynthetically available radiation was calculated as 45% of the total radiation (Dykyjova 1978; Wetzel 1975). Depth integrated gross production was expressed as grams oxygen per meter square per day by computing the data obtained at 3 hourly intervals from dawn to dusk at two levels. Production values are also expressed as grams carbon and kilo calories per meter square per day, following Vollenweider (1971) and Brody (1945) and photosynthetic efficiency is expressed as the percentage of energy utilized from the photosynthetically available radiation.

4. Results

The diurnal variation in gross production, net production and respiratory consumption during the period of study is presented in figure 2 and their mean values during different seasons in tables 1 and 2.

4.1 *Gross production*

The gross production values recorded during the period of study ranged from 0·10 mg $O_2l^{-1}h^{-1}$ (January 1984) to 1·40 mg $O_2l^{-1}h^{-1}$ (September 1983) at the surface and from 0·06 mg $O_2l^{-1}h^{-1}$ (September 1983) to 0·73 mg $O_2l^{-1}h^{-1}$ (February 1984) in water at 1 m depth. The annual mean values of gross production ranged from 0·230 ± 0·086 to 0·656 ± 0·343 mg $O_2l^{-1}h^{-1}$ in surface water and from 0·272 ± 0·142 to 0·383 ± 0·169 mg $O_2l^{-1}h^{-1}$ in water at 1 m depth. Peak values of gross production occurred between 9–12 h or from 12–15 h during the different months at both the levels. The minimum values, however, were recorded between 6–9 h in surface water, while at 1 m depth they occurred during different times of the day in the different months (figure 2).

Higher values of gross production were recorded from 6–12 h in summer and from 12–18 h during monsoon in surface water (table 1), while in water at 1 m depth higher values of gross production were recorded at 15 h in summer and from 15–18 h in monsoon (table 2). Values of coefficient of variation for this parameter are higher in water at 1 m depth than at the surface.

4.2 *Net production*

The net production values ranged from 0·06 mg $O_2l^{-1}h^{-1}$ (September, November 1983 and January, February, April, July 1984) to 0·66 mg $O_2l^{-1}h^{-1}$ (September 1983 and February 1984) in surface water and from 0·0 mg $O_2l^{-1}h^{-1}$ (September

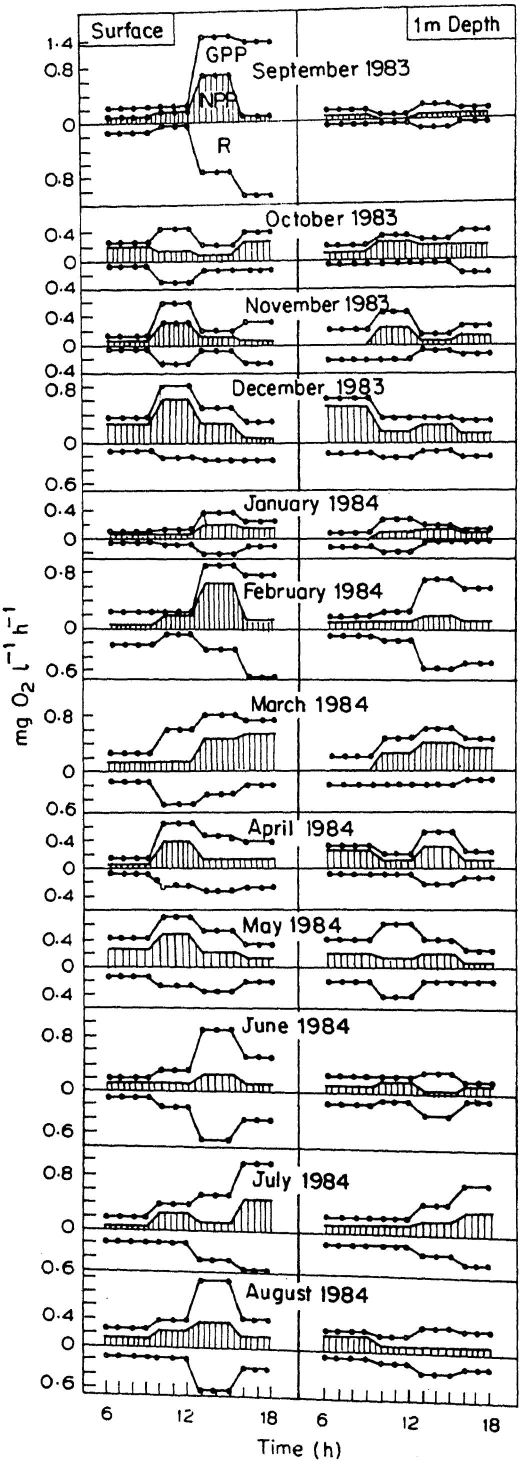

Figure 2. Diurnal variation in the phytoplankton production of lake Kondakarla-monthly changes from September 1983 to February 1984 (GPP, gross primary production; NPP, net primary production; R, respiration).

Table 1. Diurnal variation in the phytoplankton production (mg $O_2 l^{-1} h^{-1}$) in surface water of lake Kondakarla.

Period of study		Time (h)											
		6–9			9–12			12–15			15–18		
		GPP	NPP	RC	GPP	NPP	RC	GPP	NPP	RC	GPP	NPP	RC
Winter	$\bar{X}$	0·212	0·110	0·102	0·455	0·305	0·150	0·507	0·312	0·195	0·422	0·102	0·320
	± SD	0·104	0·086	0·040	0·276	0·210	0·088	0·266	0·205	0·076	0·219	0·043	0·212
	CV (%)	49·06	78·72	58·68	60·71	69·02	49·06	52·56	65·86	39·13	51·91	42·73	66·25
Summer	$\bar{X}$	0·247	0·145	0·102	0·580	0·280	0·300	0·680	0·262	0·418	0·497	0·230	0·267
	± SD	0·099	0·072	0·032	0·151	0·151	0·101	0·192	0·122	0·145	0·152	0·173	0·081
	CV (%)	40·13	49·85	31·89	26·11	54·10	33·91	28·26	46·84	34·93	30·59	75·30	30·52
Monsoon	$\bar{X}$	0·230	0·112	0·118	0·365	0·195	0·170	0·782	0·312	0·470	0·797	0·245	0·552
	± SD	0·030	0·058	0·033	0·098	0·065	0·096	0·455	0·237	0·225	0·386	0·143	0·438
	CV (%)	13·04	51·59	28·46	26·94	33·33	56·87	58·27	75·97	48·00	48·42	58·68	79·44
Annual	$\bar{X}$	0·230	0·122	0·108	0·466	0·260	0·206	0·656	0·295	0·361	0·572	0·192	0·380
	± SD	0·086	0·074	0·044	0·204	0·161	0·116	0·343	0·196	0·200	0·315	0·147	0·311
	CV (%)	37·44	61·19	41·44	44·99	62·07	56·44	52·36	66·33	55·57	55·15	76·38	81·86

GPP, Gross primary production; NPP, net primary production; RC, respiratory consumption.

Table 2. Diurnal variation in the phytoplankton production (mg $O_2 l^{-1} h^{-1}$) in waters at 1 m depth of lake Kondakarla.

Period of study		Time (h)											
		6–9			9–12			12–15			15–18		
		GPP	NPP	RC	GPP	NPP	RC	GPP	NPP	RC	GPP	NPP	RC
Winter	$\bar{X}$	0·290	0·165	0·125	0·345	0·170	0·175	0·355	0·170	0·185	0·330	0·122	0·208
	±SD	0·217	0·217	0·048	0·075	0·053	0·028	0·232	0·072	0·200	0·171	0·012	0·163
	CV (%)	74·99	131·70	38·57	21·83	31·40	16·41	65·35	42·82	108·27	52·00	10·60	78·62
Summer	$\bar{X}$	0·297	0·147	0·150	0·362	0·180	0·182	0·465	0·247	0·218	0·295	0·162	0·133
	±SD	0·074	0·096	0·054	0·136	0·054	0·137	0·105	0·129	0·030	0·098	0·100	0·046
	CV (%)	25·19	65·62	36·20	37·64	30·17	75·16	22·78	52·47	13·93	33·34	62·05	34·73
Monsoon	$\bar{X}$	0·230	0·145	0·085	0·227	0·130	0·097	0·330	0·147	0·183	0·397	0·180	0·217
	±SD	0·073	0·072	0·025	0·110	0·091	0·032	0·104	0·058	0·087	0·216	0·099	0·117
	CV (%)	32·09	49·85	30·56	44·32	70·71	33·52	31·63	39·34	47·82	54·34	55·41	54·24
Annual	$\bar{X}$	0·272	0·152	0·120	0·311	0·160	0·151	0·383	0·188	0·195	0·340	0·155	0·185
	±SD	0·142	0·143	0·051	0·122	0·072	0·091	0·169	0·101	0·128	0·174	0·085	0·125
	CV (%)	52·37	94·31	43·30	39·40	45·14	60·35	44·23	54·01	65·82	51·16	55·27	67·33

1983, January and March 1984) to 0·53 mg O_2l^{-1} h^{-1} (December 1983) at 1 m depth (figure 2).

Values of net production are maximum in summer during 6–9 h, in winter during 9–12 h and during monsoon from 12–18 h in the surface water (table 1), while in water at 1 m depth maximum values occurred in winter during 6–9 h, in summer during 9–12 h and in monsoon during 15–18 h (table 2).

4.3 *Respiratory consumption*

The values of respiratory consumption during the day showed considerable variation, with maximum values occurring between 6–9 and 12–18 h during monsoon and between 9–12 h in summer in surface water (table 1), while in water at 1 m depth these values are higher from 6–15 h during summer and from 15–18 h in monsoon (table 2).

4.4 *Seasonal variation in phytoplankton production*

The gross and net production values showed considerable variation during the study period and the data is presented in figure 3. The gross primary production values

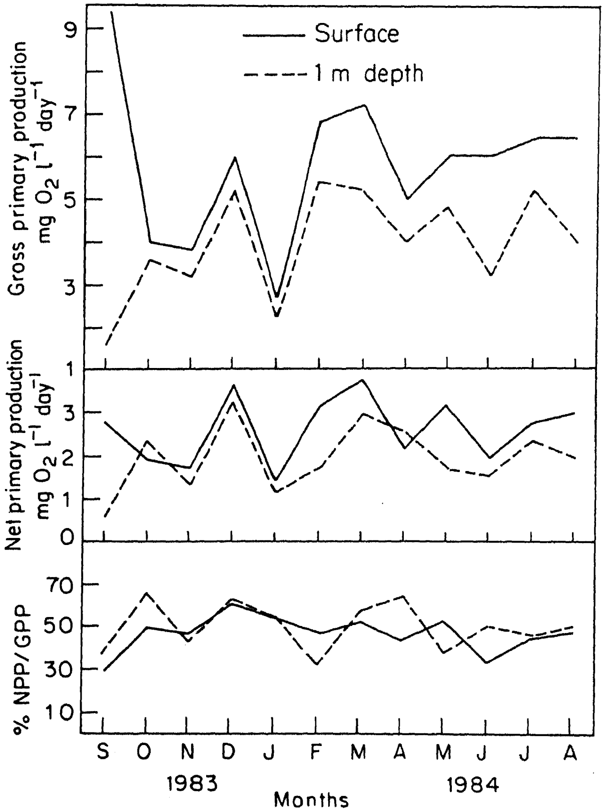

Figure 3. Seasonal variation in the phytoplankton production of lake Kondakarla.

ranged from 2·70 mg O_2l^{-1} day^{-1} during January 1984 to 9·42 mg O_2l^{-1} day^{-1} in September 1983 with a mean value of 5·81 ± 1·69 mg O_2l^{-1} day^{-1} for the surface waters. In waters at 1 m depth these values ranged from 1·60 mg O_2l^{-1} day^{-1} during September 1983 to 5·40 mg O_2l^{-1} day^{-1} in February 1984, with a mean value of 3·96 ± 1·20 mg O_2l^{-1} day^{-1}. The values of net primary production in the surface water varied from 1·50 mg O_2l^{-1} day^{-1} in January 1984 to 3·80 mg O_2l^{-1} day^{-1} during March 1984 with a mean value of 2·66 ± 0·72 mg O_2l^{-1} day^{-1}, while at 1 m depth these values ranged from 0·60 mg O_2l^{-1} day^{-1} during September 1983 to 3·30 mg O_2l^{-1} day^{-1} in December 1983 with an annual average of 2·00 ± 0·73 mg O_2l^{-1} day^{-1}. The percentage of net/gross production ranged from 29·72 (September 1983) to 61·66 (December 1983) in surface water and from 33·33 (February 1984) to 66·66 (October 1983) in waters at 1 m depth.

4.5 *Depth integrated production and photosynthetic efficiency*

The depth integrated production varied from 4·08 g O_2m^{-2} day^{-1} on 29th January 1984 to 13·05 g O_2m^{-2} day^{-1} on 18th September 1983 (1·276 and 4·074 g C m^{-2} day^{-1} respectively). The photosynthetic efficiency varied from 0·580% on 9th April 1984 to 4·301% on 15th August 1984. In the remaining months photosynthetic efficiency of the lake waters varied from 0·617–2·274% when the total radiation was found to vary between 3556·49–5830·77 kcal m^{-2} day^{-1} (table 3).

5. Discussion

Wetzel (1975) observed that the evaluation of the methodology of oxygen changes or carbon uptake for measuring *in situ* rates of production of phytoplankton has shown that the diurnal periodicity of photosynthesis often is not proportional to the daily

Table 3. Solar radiation, phytoplankton production (kcal m^{-2} day^{-1}) and photosynthetic efficiency (%) of lake Kondakarla.

Year and month	Total solar radiation (kcal m^{-2} day^{-1})	Photosynthetically active radiation (PAR) (kcal m^{-2} day^{-1})	Gross primary production g O_2 m^{-2} day^{-1}	Gross primary production g C m^{-2} day^{-1}	Gross primary production kcal m^{-2} day^{-1}	Photosynthetic efficiency (%)
1983						
18th Sept	4537·51	2041·87	13·05	4·074	45·67	2·236
14th Oct	5577·50	2509·88	9·30	2·904	32·55	1·296
20th Nov	3566·49	1604·92	8·11	2·533	28·38	1·768
21st Dec	4208·68	1893·91	12·31	3·843	43·08	2·274
1984						
29th Jan	5135·58	2311·01	4·08	1·276	14·28	0·617
19th Feb	5584·65	2513·01	9·57	2·988	33·49	1·332
18th Mar	5830·77	2623·84	9·91	3·095	34·68	1·321
9th Apr	6031·33	2714·09	4·50	1·404	15·75	0·580
12th May	6145·98	2765·69	4·96	1·550	17·36	0·627
17th June	2407·74	1083·48	4·59	1·435	16·06	1·482
29th July	1573·16	707·92	5·79	1·809	20·26	2·861
15th Aug	1502·39	676·07	8·31	2·595	29·08	3·301

insolation curve. Most studies (Schmidt 1973) show maximum photosynthetic rates in the morning hours, with a subsequent reduction at mid-day again followed by a recovery in the late afternoon. This diurnal periodicity is particularly acute at the surface and undoubtedly is partly related to photo-inhibition and increased rates of extra cellular release of organic photo-assimilates. However, fluctuations in the diurnal production values of lake Kondakarla reveal that the maximum production rates were recorded between 12–15 h both in surface and at 1 m depth, though during 6–9 h the productivity was much less in waters at 1 m depth. Kannan and Job (1980) observed peak production values between 9–12 h in Sathiar reservoir, while Saunders *et al* (1975) have reported maximum phytoplankton production between 10 a.m. and 12 noon in some temporary pools in Madurai. Schmidt (1973), however, observed the maximum production value between 8–10 h for a tropical lake of central Amazonia, while Naiman and Gerking (1975) observed the peak values during the afternoon for a thermal stream in California. Ohle (1958), Doty (1959), Vollenweider (1971), Nauwerck (1963) also reported great differences in the production rates between early morning and late afternoon.

Westlake (1966) suggested that the annual net primary productivity is roughly about 50% of the gross production. In the present study the percentage of net production ranged from 29·72–61·66 with an average value of 47·11 in the surface water and from 33·33–66·66 with an average value of 50·46 for waters at 1 m depth. Thus the respiratory activity of surface waters of lake Kondakarla is slightly higher than the waters at 1 m depth, contrary to the general belief that the respiratory consumption at the surface will be less than that of the deeper waters. However, in our present study the mean values of respiratory consumption of the surface waters are lower than those at 1 m depth during 6–12 h, while from 12–18 h a reverse trend has been observed with higher values of respiratory consumption at the surface rather than at 1 m depth. In other water bodies in India the respiratory consumption was reported to vary between 45·4–48·1% in Chilwa lake (Srivastava and Sahai 1976), 27·27–81·96% in sewage fed pond at Bhagalpur (Nasar and Nasar 1978) and 43·8–62·4% in Godavari river, Rajahmundry (Ganapathi 1972). Among the various water bodies of Andhra Pradesh where information is available on the primary productivity rates, the productivity of lake Kondakarla (1·276–4·074 g C m^{-2} day^{-1}) can be compared with Mir Alam lake (2·10–4·50 g C m^{-3} day^{-1}) (Zafar 1986).

Wetzel (1975) has pointed out that since computation of phytoplankton productivity must be made on an annual basis, it is necessary to look first at the magnitude of the seasonal variation. Kaul (1977) observed that in Kashmir lakes production during summer is 3 times greater than that in autumn, while in the present study no such trend has been observed at lake Kondakarla. Venu and Seshavatharam (1984) reported that depth integrated production varied from 0·74–16 g C m^{-2} day^{-1} at different sites of lake Kondakarla. However, during the period of this study the depth integrated production values are found to be much less ranging from 1·276–4·074 g C m^{-2} day^{-1}. Sumitra (1971) observed that the ratio between maximum to the minimum production ranged from 4 in Teppakulam tank to 10 in Othakodai pond, while at lake Kondakarla the values ranged between 3·19 and 3·48 at different levels. The present study lends support to the earlier views of Hulbert *et al* (1960), Menzel and Ryther (1961), Prasad and Nair (1963) and Nasar and Datta Munshi (1975) who observed that in tropical fresh waters production is moderate throughout the year as the temperature fluctuations are slight.

The photosynthetic efficiency values ranged from 0·580–4·301% at lake

Kondakarla. Sreenivasan (1968) estimated the photosynthetic efficiencies of Amaravathi and Stanley reservoirs to vary from 0·27–0·67% and 0·3–0·59% respectively. However, Ganapathi and Sreenivasan (1972) reported much higher values of photosynthetic efficiency ranging from 2·62–5·70% for several fish ponds and Fort Moats of south India, while Sreenivasan (1976) reported a range of 1·56–4·03 at Ayyakulam, Tamaraikulam and Sarvatheertham ponds.

In continuation of the present study, correlations between various physio-chemical factors and phytoplankton production of lake Kondakarla will be communicated soon in a separate paper.

Acknowledgement

The present paper is a part of the work carried out under a research project on the Limnology of lake Kondakarla sanctioned by the University Grants Commission, New Delhi to one of the authors (VS).

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 2, April 1988, pp. 111–120.
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Chemotaxonomic characters in twelve species of the genus *Pennisetum* (Poaceae)

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MS received 25 May 1987

Abstract. The present study reports some chemotaxonomic characters in 12 species of the genus *Pennisetum* (Poaceae). Distribution pattern of 3 biochemical constituents, viz. phenolic compounds, proteins and esterase isozymes were studied in the leaves of the 12 species, using paper chromatography and polyacrylamide gel electrophoresis. A total of 15 phenolic compounds, 23 protein bands and 19 esterase bands were observed in all these species. Data on these 3 chemotaxonomic characters were subjected to cluster analysis which resulted in grouping the 12 species into 5 clusters.

Keywords. Phenolics; leaf proteins; leaf esterases; chemotaxonomy.

1. Introduction

The phytochemical characters that are commonly recognized as of significance in biosystematic studies are phenols and proteins (including isoenzymes). In the genus *Pennisetum*, except for the study of phenolic constituents in *P. orientale* (Bate-Smith 1968), alcohol dehydrogenase (Banuett-Bourrillion and Donald 1979), amylases (Beleia and Marston 1981; Sheorain and Wagle 1981), peroxidase and esterases (Gupta *et al* 1980a, b) and polyphenol oxidase (Singh and Gupta 1981) in *P. americanum*, no comparable published information on the other species is available. The present work has been undertaken to study the distribution of phenolic constituents, proteins and esterases in the leaves of 12 species and to understand the species affinities as reflected by these 3 chemotaxonomic characters.

2. Materials and methods

The following 12 species were obtained in the form of culms or seeds from the Genetic Resources Unit, ICRISAT, Patancheru (India) and were nurtured under similar conditions at the Experimental Farm of the University Botany Department. *Pennisetum americanum* (L.) Leeke (=*P. typhoides* Stapf and Hubb.), *P. violaceum* Rich., *P. purpureum* Schumach., *P. mezianum* Leeke., *P. hohenackeri* Hochst ex Steud., *P. setaceum* (Forsk.) Chiov., *P. divisum* (Forsk ex Gmel) Henr., *P. orientale* L.C. Rich., *P. clandestinum* Hochst. ex Chiov., *P. villosum* R.Br. ex Fresen., *P. squamulatum* Fresen., *P. polystachion* (L.) Schult.

Leaf material was collected at random from the mid regions of the plants. The phenolic constituents were extracted following the modified method (Das and Rao 1964) of Bate-Smith (1958). Disc gel electrophoresis was performed following essentially the method of Davis (1964) and polyacrylamide gels (7·7 and 9% respectively) were used for the separation of both proteins and esterases. For esterases, the buffer system used was that of Scandalios (1969) and the staining schedule followed was that of Shaw and Prasad (1970). Electrophoresis was carried out at 6–10°C and at 150 volts for the first 15 min and then at 200 volts till the indicator dye (Bromophenol blue) migrated to the ends of the gel tubes.

The numbering of the protein and esterase bands followed the standard procedure, that is, the fastest (anodic) moving band being no. 1. Similarity indices were calculated as follows:

$$\mathrm{SI}=\frac{\text{Number of pairs of similar bands}}{\begin{matrix}\text{Number of pairs}\\ \text{of similar bands}\end{matrix}+\begin{matrix}\text{Number of}\\ \text{different bands}\end{matrix}}\times 100.$$

Data on the similarity indices determined for all the 3 chemotaxonomic characters (phenolic compounds, proteins and esterases) were together subjected to cluster analysis (Rao 1952).

3. Results and discussion

The experimental results of paper chromatography of phenolic compounds and electrophoresis of leaf proteins and esterases are described below.

3.1 *Phenolic compounds*

A total of 15 phenolic compounds were identified in the 12 species of *Pennisetum* (figure 1A). Data on the intensities of the spots and the percentage similarity between the species are presented in tables 1 and 2 respectively. From results in table 1, it can be observed that 7 species, viz. *P. americanum*, *P. purpureum*, *P. setaceum*, *P. divisum*, *P. orientale*, *P. squamulatum* and *P. polystachion*, possessed the same 12 phenolic compounds but in different intensities (figure 1B). Among the remaining 5, 3 species, viz. *P. mezianum*, *P. clandestinum* and *P. villosum*, were devoid of some of these 12 compounds, i.e. *P. mezianum* lacked cis-sinapic acid, *P. clandestinum* was devoid of iso-chlorogenic acid while *P. villosum* did not possess *p*-hydroxy benzoic acid (figure 1 C–E). In the two remaining species, *P. violaceum* and *P. hohenackeri*, in addition to some of the above 12 compounds, some other compounds were observed, viz. phloretic acid in *P. violaceum*, and homoprotocatechuic acid and protocatechuic acids in *P. hohenackeri* (figure 1F, G).

Bate-Smith (1968) studied the phenolic pattern in *P. orientale* and reported the occurrence of caffeic acid, *p*-coumaric acid and ferulic acid. In the present study both the cis and trans forms of *p*-coumaric acid and the derivatives of caffeic acid—isochlorogenic and chlorogenic acids were observed. Sinapic acid, which was reported to be absent, was detected in both cis and trans forms in the present study. This difference between the present study and that of Bate-Smith (1968) might be due to the difference in the specimens investigated since several chromosomal types with 2n = 18 to 45 are known to exist in this species.

Based on the distribution of the phenolic compounds, estimations have been made to assess the similarities amongst the 12 species (table 2). The percentage similarities between the species ranged from 69·20 in *P. violaceum* vs *P. mezianum* to 100 for several pairs of species compared. The two species *P. americanum* and *P. purpureum*

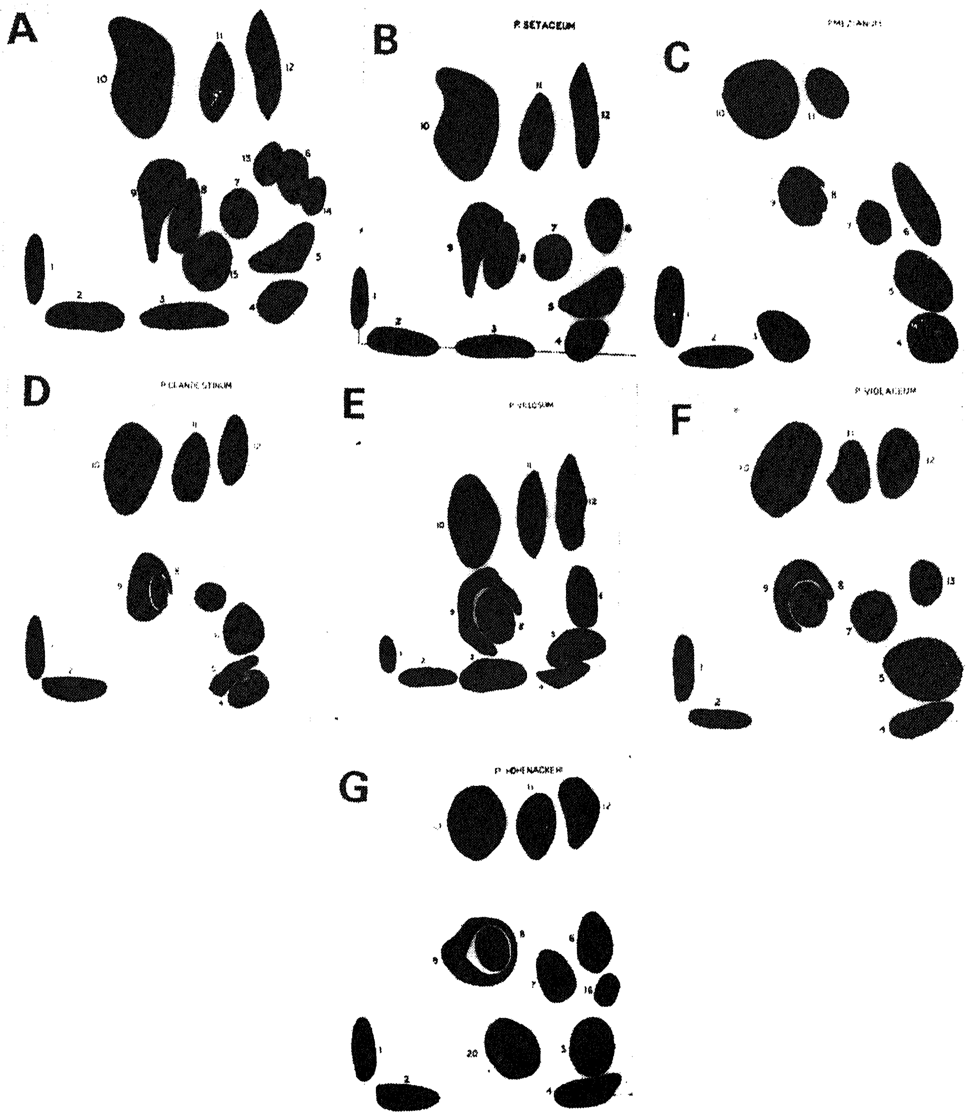


Figure 1. Two dimensional chromatographic patterns of the phenolic compounds from the leaf extracts of 12 species of *Pennisetum*. **A.** Master chromatogram showing the relative positions of 15 phenolic compounds. **B.** Chromatogram of *P. setaceum* without spots 13, 14 and 15. Same compounds are present in *P. americanum*, *P. purpureum*, *P. divisum*, *P. orientale*, *P. squamulatum* and *P. polystachion*. **C.** Chromatogram of *P. mezianum*. Spots 12–15 are absent. **D.** Chromatogram of *P. clandestinum* devoid of spots 3 and 13–15. **E.** Chromatogram of *P. villosum*. Spots 7 and 13–15 are absent. **F.** Chromatogram of *P. violaceum*. Spots 3, 6, 14 and 15 are absent and having spot 13. **G.** Chromatogram of *P. hohenackeri*, without spots 3 and 13 and having spots 14 and 15.

Key: Spot 1, Quercetin, 3-glucoside; 2, rutin; 3, isochlorogenic acid; 4, chlorogenic acid; 5, neo-chlorogenic acid; 6, cis-*p*-coumaric acid; 7, *p*-hydroxy benzoic acid; 8, scopoletin; 9, trans-*p*-coumaric acid; 10, trans sinapic acid; 11, vanillic acid; 12, cis-sinapic acid; 13, phloretic acid; 14, homo protocatechuic acid; 15, protocatechuic acid.

Table 1. Intensities of the spots of phenolic compounds in 12 species of *Pennisetum*.

Compound*	*P. americanum*	*P. violaceum*	*P. purpureum*	*P. mezianum*	*P. hohenackeri*	*P. setaceum*	*P. divisum*	*P. orientale*	*P. clandestinum*	*P. villosum*	*P. squamulatum*	*P. polystachion*
Quercetin, 3-glucoside	d	d	f	f	f	f	d	d	d	f	d	d
Rutin	d	d	f	f	f	f	d	f	d	f	f	f
Isochlorogenic acid	f	—	f	f	—	f	f	f	—	f	f	f
Chlorogenic acid	d	d	d	d	d	d	d	d	d	d	d	d
Neo-chlorogenic acid	s	s	s	d	s	s	d	s	s	d	s	d
Cis-*p*-coumaric acid	d	—	f	f	d	d	s	d	s	d	d	f
p-Hydroxybenzoic acid	d	s	f	d	s	s	d	s	d	—	d	d
Scopoletin	d	s	s	d	s	s	s	s	d	s	d	d
Trans-*p*-coumaric acid	d	d	f	f	d	d	f	d	d	d	d	f
Trans-sinapic acid	s	d	d	d	d	d	d	s	d	d	d	d
Vanillic acid	s	s	s	s	s	s	s	s	s	s	d	d
Cis-sinapic acid	s	f	f	—	d	d	f	s	f	d	f	f
Phloretic acid	—	d	—	—	—	—	—	—	—	—	—	—
Homo protocatechuic acid	—	—	—	—	f	—	—	—	—	—	—	—
Protocatechuic acid	—	—	—	—	f	—	—	—	—	—	—	—

s, Strongly present; d, distinctly present; f, faintly present; —, absent.

*First direction solvent = benzene : acetic acid : water in 6 : 7 : 3 (by volume).

Second direction solvent = sodium formate : formic acid : water in 10 : 1 : 200 (by volume).

showed 100% similarity while *P. violaceum* was showing a similarity of 76·9% with both the species. Both *P. americanum* and *P. purpureum* showed the same percentage similarities with *P. mezianum* as well as with *P. hohenackeri* while *P. violaceum* was showing less similarity with these two species. These observations are of interest when the relationships of the 3 species, *P. americanum, P. violaceum* and *P. purpureum*, are considered. *P. purpureum* is an allotetraploid (see Pantulu and Rao 1982) with one of its genomes homologous to *P. americanum.* In spite of this, data on the phenolic compounds showed 100% similarity between these two species. *P. violaceum* was considered to be the probable progenitor of the cultivar *P. americanum* based on hybridization experiments (Bruken 1977). But the relation between these two genomes is only 76%. Based on these percentage similarities, *P. violaceum* is equidistant from both *P. americanum* and *P. purpureum.*

3.2 *Leaf proteins*

The spectrum of protein fractions varied among the 12 species and 23 of them were identified (figure 2a). No protein profile was observed for *P. polystachion.* In this, though several gel concentrations and electrophoretic conditions were tried, no clear resolution of bands was observed.

The number of bands per species ranged from a minimum of 7 in *P. purpureum* to a maximum of 11 in *P. violaceum* and *P. mezianum* (figure 2a). Band no. 13 was the most common one occurring in about 10 out of the 11 species; *P. purpureum* was the only species which lacked this. Each of the band nos 23, 9, 7, 4, 3, 2 and 1 occurred in few species. Though the overall protein pattern was species specific, no particular band could be assigned to any one species.

Similarity indices based on protein patterns ranged from 12·5% as observed between *P. purpureum* vs *P. mezianum* to a maximum of 66% between the pairs- *P. setaceum* and *P. orientale* and *P. purpureum* and *P. hohenackeri* (table 2). Among the 3 species *P. americanum, P. violaceum* and *P. purpureum, P. americanum* was found to be more closer to *P. violaceum* than to *P. purpureum.* In addition to these two species *P. americanum* showed greater similarities with *P. orientale, P. squamulatum* and *P. setaceum.* At this juncture, it may be of interest to note that the only successful hybrids produced, using *P. americanum* as one of the parents, were those which involved these 5 species, *P. violaceum, P. purpureum, P. setaceum, P. orientale* and *P. squamulatum,* as the other parents. The prostrate species *P. clandestinum* is more closer to *P. divisum* and *P. orientale* than to the others.

3.3 *Leaf esterases*

A total of 19 different bands were observed when all the esterase patterns in the 12 species were studied (figure 2b). In the case of esterases also, *P. polystachion* was peculiar in showing a single zone of esterase activity and this corresponded to band no. 17 of the other species.

P. mezianum showed a maximum number of 10 bands, while *P. setaceum* had the minimum of 4. Band nos 7 and 10 were characteristic for *P. villosum* while band nos 1 and 2 were observed only in *P. clandestinum.*

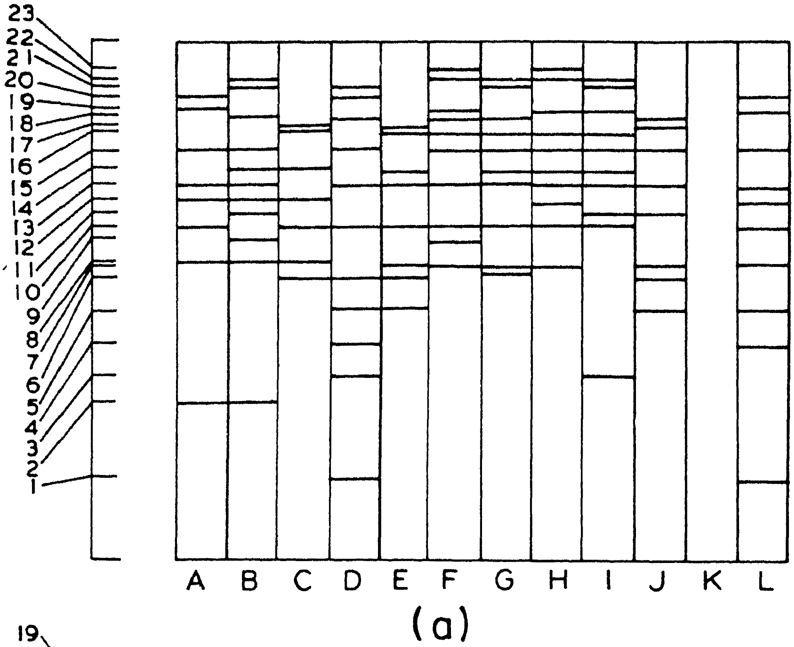
The percentage similarities as summarised in table 2 ranged from zero in species pairs

Table 2. Percentage similarities between 12 species based on the 3 chemotaxonomical characters.

Species		*P. americanum*	*P. violaceum*	*P. purpureum*	*P. mezianum*	*P. hohenackeri*	*P. setaceum*	*P. divisum*	*P. orientale*	*P. clandestinum*	*P. villosum*	*P. squamulatum*	*P. polystachion*
P. americanum	i	100·00	76·90	100·00	91·60	78·60	100·00	100·00	100·00	91·60	91·60	100·00	100·00
	ii	100·00	35·70	25·00	26·66	23·10	38·46	28·57	50·00	30·76	23·07	50·00	0·00
	iii	100·00	30·00	66·00	42·00	33·00	38·00	30·00	30·00	17·00	8·00	50·00	0·00
	iv	100·00	47·53	63·67	53·42	44·90	58·82	52·86	60·00	46·45	40·89	66·67	33·33
P. violaceum	i		100·00	76·90	69·20	76·90	76·90	76·90	76·90	91·60	69·20	76·90	76·90
	ii		100·00	20·00	22·20	18·75	40·00	50·00	33·30	33·30	35·71	16·66	0·00
	iii		100·00	27·00	7·00	0·00	25·00	9·00	20·00	30·00	18·00	22·00	17·00
	iv		100·00	41·30	32·00	31·88	47·30	45·30	43·40	51·63	40·91	38·52	31·30
P. purpureum	i			100·00	91·60	78·60	100·00	100·00	100·00	91·60	91·60	100·00	100·00
	ii			100·00	12·50	66·00	21·42	30·76	41·66	23·07	25·00	13·33	0·00
	iii			100·00	50·00	30·00	20·00	27·00	90·00	15·00	7·00	44·00	0·00
	iv			100·00	51·37	58·20	47·14	52·59	60·55	43·22	41·20	52·44	33·33
P. mezianum	i				100·00	71·40	91·60	91·60	91·60	83·30	83·30	91·60	91·60
	ii				100·00	26·66	23·52	43·75	16·66	33·30	35·71	50·00	0·00
	iii				100·00	50·00	17·00	45·00	45·00	13·00	21·00	36·00	0·00
	iv				100·00	49·35	44·04	60·12	51·09	43·20	46·67	59·20	30·53
P. hohenackeri	i					100·00	78·60	78·60	78·60	84·60	71·40	78·50	78·50
	ii					100·00	28·60	38·46	38·46	30·76	45·45	16·66	0·00
	iii					100·00	13·00	22·00	38·00	20·00	33·00	25·00	0·00
	iv					100·00	40·66	46·35	51·69	45·12	49·95	40·05	26·17
P. setaceum	i						100·00	100·00	100·00	91·60	91·60	100·00	100·00
	ii						100·00	53·85	66·60	35·71	28·57	25·00	0·00
	iii						100·00	43·00	25·00	10·00	22·00	29·00	0·00
	iv						100·00	65·62	63·87	45·77	47·39	51·33	33·33

P. divisum	i	100·00	100·00	91·60	91·60	100·00	100·00
	ii	100·00	53·85	46·15	28·57	25·00	0·00
	iii	100·00	50·00	0·00	44·00	38·00	0·00
	iv	100·00	67·95	45·92	54·72	54·33	33·33
P. orientale	i		100·00	91·60	91·60	100·00	100·00
	ii		100·00	46·15	20·00	33·30	0·00
	iii		100·00	8·00	30·00	57·00	0·00
	iv		100·00	48·50	47·20	63·43	33·33
P. clandestinum	i			100·00	83·30	91·60	91·60
	ii			100·00	21·42	26·60	0·00
	iii			100·00	0·00	9·00	0·00
	iv			100·00	34·91	42·40	30·53
P. villosum	i				100·00	91·60	91·60
	ii				100·00	20·00	0·00
	iii				100·00	20·00	14·00
	iv				100·00	43·87	35·20
P. squamulatum	i					100·00	100·00
	ii					100·00	0·00
	iii					100·00	0·00
	iv					100·00	33·33
P. polystachion	i						100·00
	ii						100·00
	iii						100·00
	iv						100·00

i, Leaf phenolic compounds; ii, leaf proteins; iii, leaf esterases; iv, average of i, ii and iii.



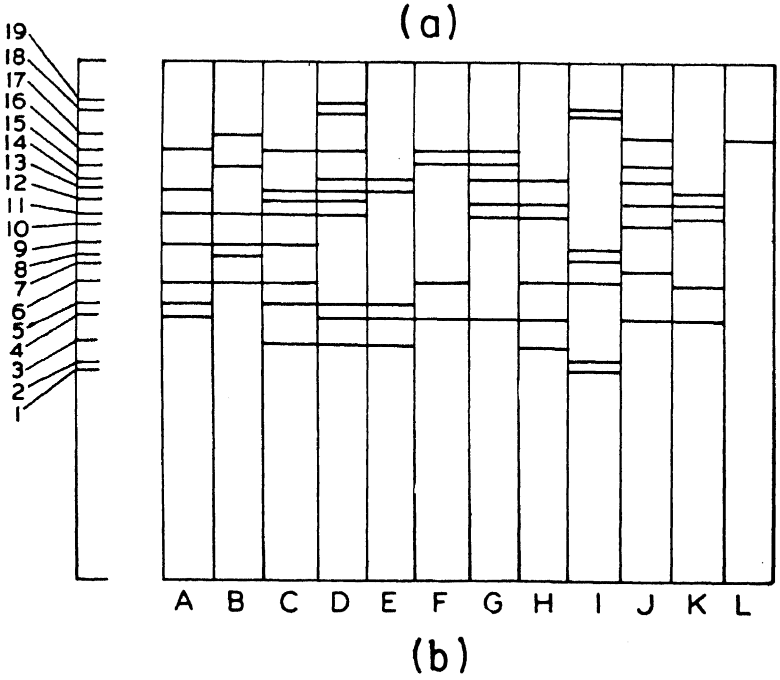


Figure 2. Schematic representation of protein and esterase patterns in the species of *Pennisetum*. **a.** Leaf proteins, A, *P. americanum*; B, *P. violaceum*; C, *P. purpureum*; D, *P. mezianum*; E, *P. hohenackeri*; F, *P. setaceum*; G, *P. divisum*; H, *P. orientale*; I, *P. clandestinum*; J, *P. villosum*; K, *P. polystachion*; L, *P. squamulatum*. **b.** Leaf esterases, A–J, Same as in a; K, *P. squamulatum*; L, *P. polystachion*.

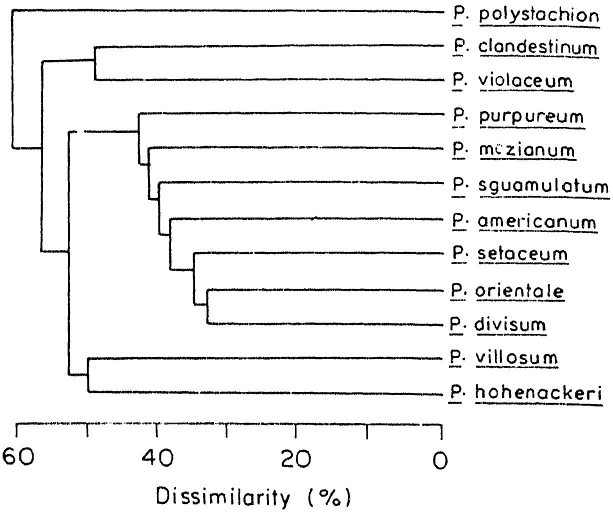


Figure 3. Phenogram based on chemotaxonomy showing 4 clusters.

P. violaceum vs *P. hohenackeri*, *P. divisum* vs *P. clandestinum* and *P. clandestinum* vs *P. villosum*, to 66% observed between *P. americanum* and *P. purpureum*.

Based on the similarity indices it can be observed that *P. americanum* was closer to *P. purpureum* than to any other species. Likewise *P. violaceum* was nearer to *P. americanum* than to *P. purpureum*. The two species *P. violaceum* and *P. clandestinum* showed very low percentage similarities with the other species.

On the basis of the information obtained now on the 3 different chemotaxonomic aspects, a cluster analysis was done and the results are presented in table 2. On the basis of such an analysis, all the 12 species could be recognized into 5 clusters (figure 3). Cluster I was the largest and included 7 species, viz. *P. divisum*, *P. orientale*, *P. setaceum*, *P. americanum*, *P. squamulatum*, *P. purpureum* and *P. mezianum*. Cluster II comprised two species, *P. violaceum* and *P. clandestinum*. The remaining 3 clusters (III, IV and V) were single-membered and included *P. hohenackeri*, *P. villosum* and *P. polystachion* respectively. It is interesting to note that *P. americanum* and its probable progenitor *P. violaceum* are separable into two clusters. *P. polystachion*, alone formed one cluster and thus seems to retain its distinctness even from the chemotaxonomic considerations, and thereby justifying its separation from the remaining species and inclusion in a separate section *Brevivalvula* by Stapf and Hubbard (1934).

Acknowledgements

The authors thank Dr M H Mengesha and Dr S Appa Rao, ICRISAT, Patancheru (India) for the supply of culms/seeds of the species and to Dr K Narayana Rao, Botany Department, SV University, Tirupati, for his help in the identification of the phenolic compounds. Financial assistance from the Council of Scientific and Industrial Research (to DMS) and the University Grants Commission (to YSR), New Delhi is gratefully acknowledged.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 2, April 1988, pp. 121–133.
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Architectural plasticity of two bamboo species (*Nehouzeua dulloa* A Camus and *Dendrocalamus hamiltonii* Nees and Arn.) in successional environments in north-east India

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MS received 4 September 1987

Abstract. Two bamboo species, *Neohouzeua dulloa* A Camus and *Dendrocalamus hamiltonii* Nees and Arn. show plasticity in architecture, biomass and nutrient allocation patterns over a successional gradient of up to 60 yr for the former and 25 yr for the latter. However, optimal biomass accumulation rate was attained in a 15 yr old fallow for both species. Maximum elongation and thickness of the zero-order branch (main axis) is attained in a 15 yr old fallow for *Neohouzeua dulloa* and in a 25 yr old fallow for *Dendrocalamus hamiltonii.* The lower order branches produced on the zero-order branch of *Neohouzeua dulloa* are generally sylleptic whereas those of *Dendrocalamus hamiltonii* are proleptic. Maximum elongation and thickness for the zero-order branch, and least bud dormancy on them occurred in a 15 yr old fallow for *Neohouzeua dulloa* and in a 25 yr old fallow for *Dendrocalamus hamiltonii. Dendrocalamus hamiltonii* in a 5 yr old fallow had a well developed first-order branch system with diffusely placed lower order branch clusters appearing as if borne on the first-order branch, unlike in older fallows where first-order branch production was continuous and lower order branch clusters appeared as if borne on the zero-order branch itself. In older fallows both species tend to allocate more biomass and nutrients to the below-ground rhizomatous component, as an adaptation for survival and vegetative regeneration after slash and burn disturbance. The growth and architecture of both species are generally geared to capitalize upon the high light regime of the early successional environment, but with a limited ability by *Neohouzeua dulloa* to tolerate shade.

Keywords. Bamboos; architecture; growth pattern; allocation pattern; secondary succession.

1. Introduction

During secondary succession after slash and burn agriculture in north-east India, edaphic (Ramakrishnan and Toky 1981; Toky and Ramakrishnan 1983b) and micro-environmental changes such as reduction in light availability occur with the development of a closed community (Singh and Ramakrishnan 1982; Toky and Ramakrishnan 1983a). Species of the early successional environment show varying degrees of plasticity to such fast changing situations. Canopy architecture that is adapted to changing light environment during succession (Boojh and Ramakrishnan 1982a, b; Shukla and Ramakrishnan 1984, 1986) could be highly plastic. Much of the work on canopy architecture in relation to succession pertains to temperate broad-leaved tree species (Brunig 1976; Kramer and Kozlowski 1979; Borchert and Slade 1981; Nelson *et al* 1981). It is only in recent times that tropical woody species have started receiving some attention (Ramakrishnan and Shukla 1982; Shukla and Ramakrishnan 1984, 1986). However, all these studies refer to different species over a successional gradient. No information is available on architectural plasticity of the same species in such a gradient. Further, information on bamboos are largely qualitative (Hallé *et al* 1978).

The present paper deals with the architectural plasticity and biomass and nutrient allocation patterns of two early successional bamboo species, *Neohouzeua dulloa* A Camus and *Dendrocalamus hamiltonii* Nees and Arn. The former is more plastic occurring in successional fallows of upto 60 yr whereas the latter is restricted to the first 25 yr of secondary succession after slash and burn agriculture (Ramakrishnan *et al* 1981; Toky and Ramakrishnan 1983a) at lower elevations in Meghalaya in north-east India.

2. Study area

The study was carried out at Lailad (200 m altitude), situated in the Khasi hills of Meghalaya, about 75 km north of Shillong (26°N and 91°5'E). The precambrian rocks are represented by gneiss, schists and granites. The soil is a red sandy loam of laterite origin, with pH 5–7. The angle of the slope generally ranged from 20–40°.

The climate has 3 distinct seasons: (i) a brief summer extending from mid-February to May, (ii) a rainy season from May to September and (iii) a mild and relatively dry winter from October to mid-February. The average maximum temperature during the summer period was 30·7°C and the average minimum was 10·8°C. Seventy three per cent of the total annual rainfall of 1800 mm occurred during the rainy season. The average maximum temperature during the rainy season was 31·4°C and the average minimum was 24·1°C, whilst that for winter months were 26·5 and 14·4°C, respectively.

3. Methods of study

Fallows of 1, 5, 15, 25 and 60 yr old were identified based on our own observations and the records available with the village headman and the Forest Department. Care was taken to ensure similar topography, soil type, exposure and slope.

3.1 *Architectural studies*

Culms are produced in both species during the monsoon season. These culms of *D. hamiltonii* would produce branches and leaves only during the next monsoon season. Hence the culms produced in June 1982 and 1983 were separately tagged. *N. dulloa* produce branches and leaves during the same growing season when the culms are initiated. In this case, the culms produced in April 1983 alone were tagged. For both species, 10 replicate culms from different clumps selected randomly were considered. Observations from a raised platform were made at weekly intervals during the period of culm elongation. When the growth slowed down, these observations were made at monthly intervals. These observations on the two species made in 5–25 yr old fallows pertained to: (i) elongation of the branches of different orders, (ii) branch production, (iii) internodal length and (iv) bud dynamics on the main axis (zero-order branch). Observations on *N. dulloa* were also made in a 60 yr old fallow.

3.2 *Allocation studies*

N. dulloa is a gregariously flowering bamboo species, that flowered last in 1968 in the study area, before total death. Therefore the entire population under observation in 1983 had a stock age of 15 yr. *D. hamiltonii* has both sporadic and gregarious flowering habits. The last gregarious flowering in the study area was in 1956. The stock age of this species in 1983 was, therefore, not more than 30 yr. This inference is also based on the very short viability for the seeds which necessitates that they should germinate soon after dispersal.

The age of a clump was measured as regeneration age which was equivalent to fallow age upto 15 yr in *N. dulloa*. Regeneration age for *D. hamiltonii* may be the same or even less than the fallow age depending upon whether the clump arose after gregarious flowering or a subsequent sporadic flowering. The population of *N. dulloa* present in 25 and 60 yr old fallows, however, would have the same stock/regeneration age of 15 yr. With uniform stock age, the clumps of *N. dulloa* in a given fallow were all of the same size. This permitted random selection of clumps. On the other hand, *D. hamiltonii* with possible differences in stock age because of gregarious and sporadic flowering had clumps of different sizes. Therefore clumps of similar size were selected in a given fallow.

Ten replicate clumps of the two species selected from each site were harvested in August 1984. The above-ground parts were separated into main axis (zero-order branch), branches (all orders except zero-order branch) and leaves. Below-ground parts (rhizome and roots) were carefully excavated, washed thoroughly and weighed. Samples were dried at 80°C and dry weights computed.

All the samples were ground and passed through a 0·5 mm sieve and analysed for elemental composition after acid digestion using methods suggested by Allen *et al* (1974). Thus, nitrogen was estimated by Kjeldahl method, phosphorus colorimetrically by ammonium molybdate blue method, potassium by flame-photometry and calcium and magnesium by EDTA-titration method.

The results were subjected to one way analysis of variance. The mean ± S.E. is a pooled estimate over the mean monthly observations with ten replications.

4. Results

4.1 *Architectural studies*

N. dulloa and *D. hamiltonii* have extensive sympodially branched under-ground rhizomes. Each branch 9–14 cm long in *N. dulloa* and 12–24 cm in *D. hamiltonii* has a narrow neck which broadens out into a thick base (figure 1d). Rhizomatous branches are produced from two of the lateral buds whereas the apical bud would give rise to the above-ground shoot system. With thinner rhizome and aerial branch systems in *N. dulloa* the clumps are more loosely organized in this species than in *D. hamiltonii*.

The crown shape of *D. hamiltonii* is highly variable. In a 5 yr old fallow because of predominantly herbaceous vegetation and the consequent better light availability, the crown is broader, tapering towards the tip (figure 1a). In older fallows, where other larger species compete for aerial space, the crown of *D. hamiltonii* becomes cylindrical in shape with a whip like tip (figure 1b). In *N. dulloa*, the crown form is more uniform in all the fallows, being broader in the middle and tapering on either side (figure 1c).

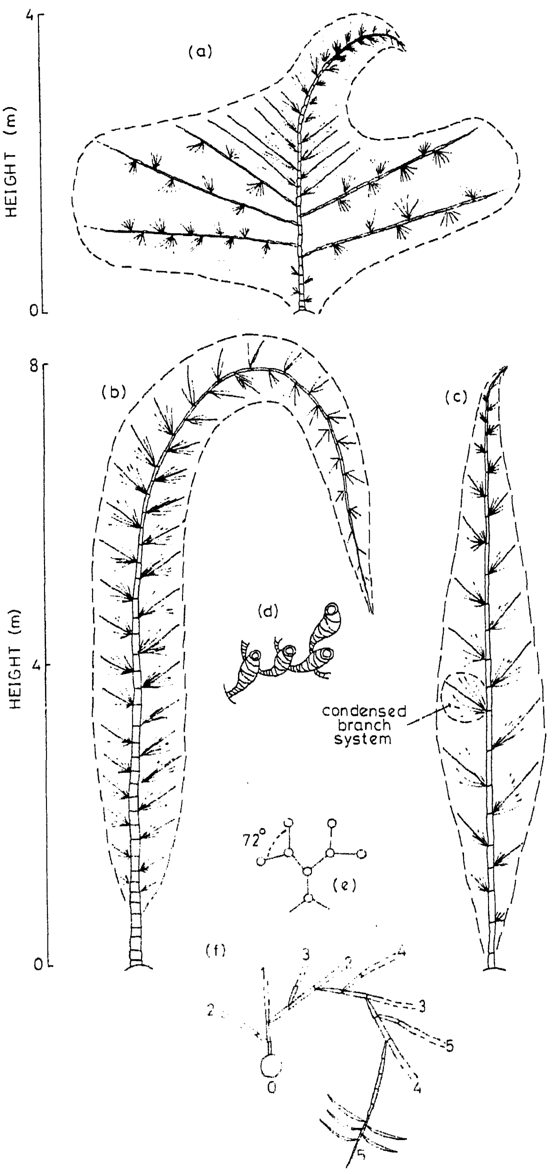

Figure 1. Canopy architecture of *D. hamiltonii* in a 5 yr old fallow (**a**), in a 15 yr old fallow (**b**) and that of *N. dulloa* in a 15 yr old fallow (**c**), Rhizome architecture of the two species (**d**) alongwith a diagramatic representation (**e**). Diagramatic representation of the organization of branch orders in a branch complement in the two bamboo species. Zero to fifth-order branches are shown alongwith the leafy shoots (**e**).

Branch production in both species is determinate. The orthotrophic zero-order branches of the two species complete their elongation within 90–120 days after initiation (figure 2) through syllepsis (in the same year) in *N. dulloa* but through prolepsis in *D. hamiltonii* (in the following year only). Reiteration (Hallé *et al* 1978) of the zero-order branch may occur in *D. hamiltonii* only. In *N. dulloa*, damage to the

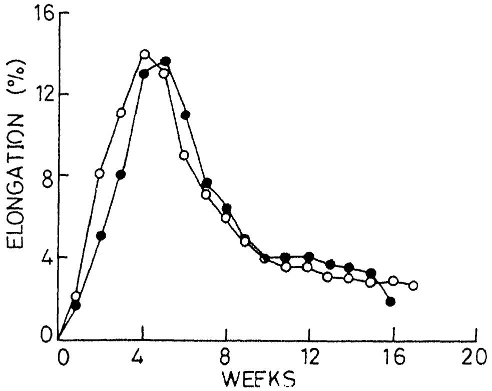

Figure 2. Elongation rate of the zero-order branch of *N. dulloa* (●) and *D. hamiltonii* (○) in a 15 yr old fallow.

apical bud would arrest further growth. All the branches on the zero-order are produced and elongation completed simultaneously. *N. dulloa* would normally produce branches upto the fourth-order except in a 15 yr old fallow where the fifth order branches may be produced, but in the subsequent year only. *D. hamiltonii* would normally produce branches upto the fifth-order. Branches in the two species are produced continuously on all nodes except in *D. hamiltonii* growing in a 5 yr old fallow, where branching was diffuse (figure 1a). The first-order branches and the subsequent orders all look as if arising in a cluster from the same point from a given node on the zero-order axis. However, each branch order is a condensed axis, one arising on the other. Thus, the axillary buds of the two basal nodes of the first-order branch would produce two second-order branches, similarly the second-order branch would produce the third-order branches and so on, so that a maximum of 31 units may be produced in a condensed form (figure 1f). However, branch production may stop at the first-order stage (one unit only), at the second-order stage (3 units only), at the third-order stage (7 units only), or at the fourth-order stage (15 units only).

The two species conform to the Mc Clure's model as described by Hallé *et al* (1978). The essential feature of this model is that all the aerial parts are vegetatively determinant in their growth. They consider the determinant axis and the branch systems to be a determinant organ, something like an elaborate compound leaf.

Leaves on both species are borne on all branches towards their tip, except the zero-order branch. These branches are shed off every year and new leafy shoots arise in their place simultaniously in *N. dulloa*, but after a brief dormancy of upto 60 days in *D. hamiltonii*. While *N. dulloa* was evergreen leaf exchanging type, *D. hamiltonii* was deciduous. Growth parameters reached its maximum in a 15 yr old fallow for *N. dulloa* ($P<0{\cdot}05$) and in a 25 yr old fallow ($P<0{\cdot}05$) for *D. hamiltonii* (table 1). Sylleptic bud dormancy in *N. dulloa* was minimal (6%) in a 15 yr old fallow whereas the proleptic (previous year's) buds of *D. hamiltonii* had declining bud dormancy with fallow age upto 25 yrs (figure 3).

The elongation of the first-order branch presented in table 2 shows that the variation in *D. hamiltonii* in a 5 yr old fallow was very high. This high variation in *D. hamiltonii* is evident from the larger differences in the elongation of the first-order

Table 1. Important characteristics (mean ± SE) of *N. dulloa* and *D. hamiltonii* in 5–60 yr old fallows.

Parameters	Fallow age (years) 5	15	25	60
Rhizomatous branch elongation (cm/yr)	9·7 ± 0·63 (12·7 ± 1·14)	13·5 ± 1·23 (21·5 ± 1·77)	11·2 ± 0·82 (23·6 ± 1·93)	9·5 ± 0·92
Clump area (cm²)	0·41 ± 0·03 (1·16 ± 0·11)	1·21 ± 0·11 (2·27 ± 0·17)	0·61 ± 0·05 (2·53 ± 0·21)	0·30 ± 0·03
Number of culms/clump	19 ± 1·30 (32 ± 2·85)	51 ± 3·20 (63 ± 4·11)	42 ± 2·90 (63 ± 2·53)	29 ± 1·90
Number of culms/ha	2000 (2200)	4600 (6000)	3300 (7300)	3000
Culm length (m)	1·35 ± 0·12 (3·80 ± 0·35)	8·30 ± 0·82 (13·70 ± 1·23)	2·90 ± 0·21 (16·90 ± 1·68)	2·05 ± 0·16
Culm diameter at 10 cm from base (cm)	2·5 ± 0·20 (5·9 ± 0·58)	5·2 ± 0·38 (14·2 ± 1·14)	2·9 ± 0·21 (16·8 ± 1·36)	2·5 ± 0·16
Annual elongation in current year's culm (cm)	145 ± 12·9 (400 ± 28·6)	835 ± 50·8 (1420 ± 73·8)	300 ± 25·8 (1750 ± 101·3)	215 ± 17·9
Total height to crown width ratio	1·69 (0·91)	6·91 (10·60)	3·22 (13·00)	2·56

Values in parentheses are for *D. hamiltonii*.

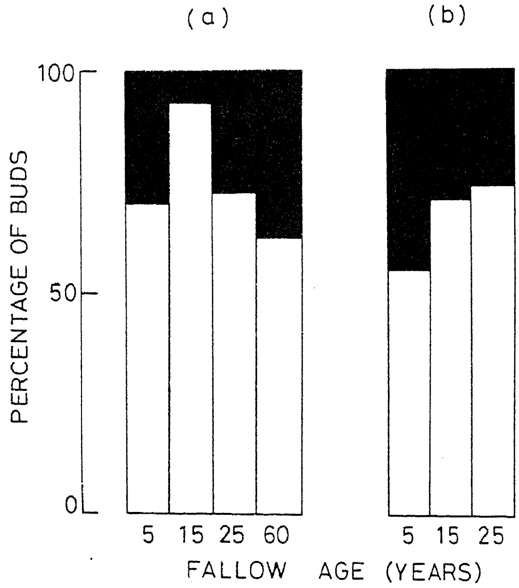

Figure 3. Variation in the dormancy of buds (%) on the current year's culm (zero-order branch) of *N. dulloa* (**a**) and last year's culm of *D. hamiltonii* (**b**), with fallow age. (■), Dormant buds; (□), active buds.

branches at different canopy positions (table 3). *N. dulloa* had, however, maximum elongation in a 15 yr old fallow and *D. hamiltonii* in a 25 yr old one. The elongation pattern for the lower order branches was similar (hence not presented here) except that elongation decreased in higher order branches.

Table 2. Variation in first-order branch elongation (cm) (mean ± SE) of two bamboos in 5–60 yr old fallows.

	Fallow age (years)			
Species	5	15	25	60
N. dulloa	40·1 ± 3·57	65·3 ± 4·00	35·2 ± 3·07	30·9 ± 3·00
D. hamiltonii	71·3 ± 20·50	65·7 ± 9·60	60·3 ± 9·70	

Table 3. Variation in elongation (cm) (mean ± SE) in first-order branches of *D. hamiltonii* at different canopy positions on zero-order branch in 5–25 yr old fallow.

Fallow age (years)	Canopy position (node number from base)			
	0–10	10–20	20–30	< 30
5	14·3 ± 0·92	254·1 ± 10·7	25·3 ± 2·6	
15	13·9 ± 0·98	75·3 ± 4·2	51·6 ± 4·7	18·7 ± 1·6
25	12·6 ± 0·81	73·9 ± 5·6	59·2 ± 2·9	23·6 ± 1·8

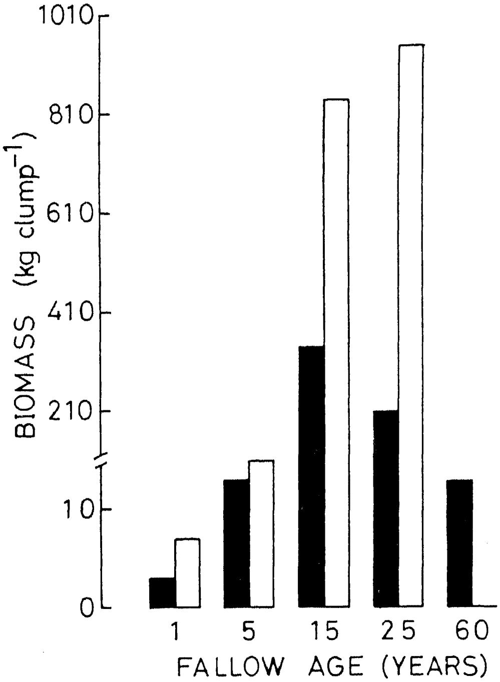

Figure 4. Standing biomass (kg clump^{-1}) of bamboos in successional fallows. (■), *N. dulloa*; (□), *D. hamiltonii*.

4.2 *Allocation studies*

The total standing biomass per clump was maximum ($P < 0{\cdot}05$) in a 15 yr old fallow for *N. dulloa* and in a 25 yr old fallow for *D. hamiltonii* (figure 4). However, the rate of accumulation for the below-ground and above-ground organs peaked in a 15 yr old fallow, for both species (figure 5). It may be noted here that the rate of accumulation in *N. dulloa* in 25 and 60 yr old fallows, was calculated on the assumption that the age of the clump was 15 yr, based upon the previous gregarious flowering.

The above-ground/below-ground ratio of *N. dulloa* declined significantly ($P < 0.05$) in a 5 yr old fallow (table 4) compared to a 1 and 15 yr old ones. This ratio was significantly lower ($P < 0.05$) in a 60 yr old fallow as compared with 15 and 25 yr. The ratio for *D. hamiltonii* declined sharply ($P < 0.05$) in 15 and 25 yr old fallows, compared to younger ones.

The allocation of biomass to the below-ground organs in *N. dulloa* was higher in 5 and 60 yr old fallows (figure 6a). In *D. hamiltonii*, 1 yr old fallow had maximum biomass allocation ($P < 0.05$) to the zero-order branch whereas the lower branch orders had more allocation ($P < 0.01$) in a 5 yr old fallow. Nitrogen allocation to the leaf component in *N. dulloa* increased ($P < 0.01$) with fallow age but decreased in the case of the zero-order branch (figure 6b). Allocation of nitrogen to the zero-order branch of *D. hamiltonii* was minimal and to the leaf component was maximal in a 5 yr old fallow. In general nitrogen allocation to the leaf component was higher ($P < 0.01$) than biomass allocation. Phosphorus allocation to the below-ground parts of both species increased ($P < 0.05$) and to the zero-order branches decreased in older fallows (figure 6c). The leaves of *N. dulloa* had more phosphorus ($P < 0.01$) allocation

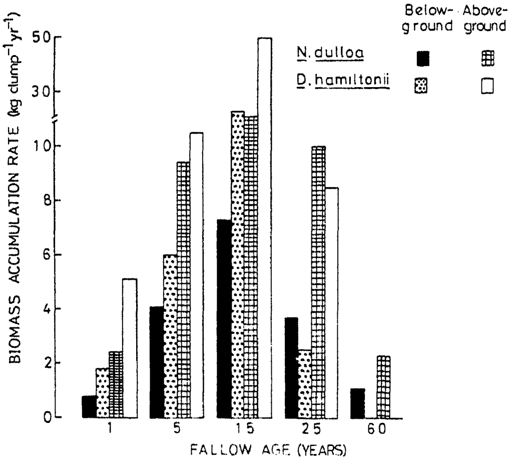

Figure 5. Biomass accumulation rates (kg clump^{-1} yr^{-1}) in above-ground and below-ground components of two bamboos in successional fallows.

Table 4. Above-ground/below-ground ratio of bamboos in 5–60 yr old fallows.

Fallow age (years)	*N. dulloa*	*D. hamiltonii*
1	2·89	2·76
5	2·34	2·69
15	2·77	2·13
25	2·75	2·24
60	2·15	—

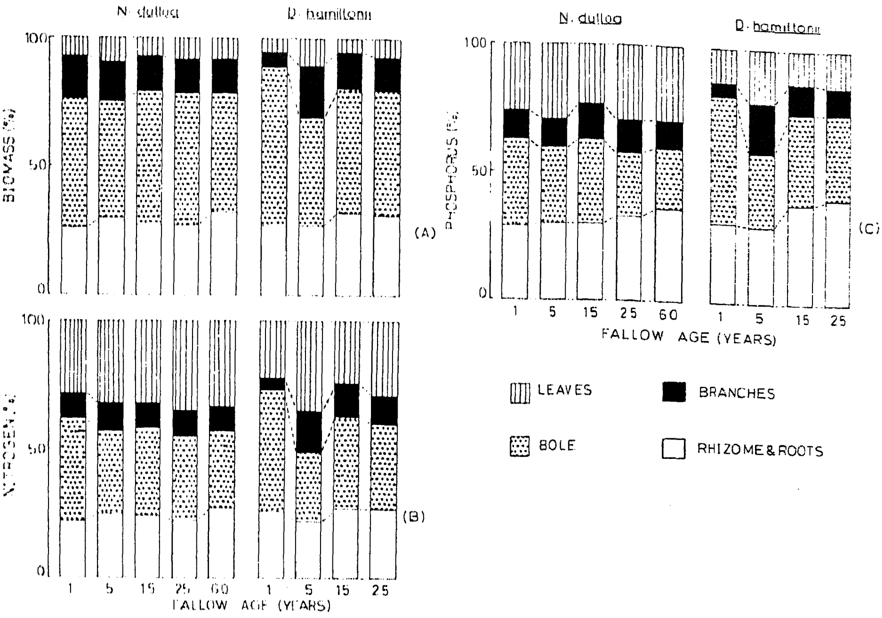

Figure 6. Allocation pattern (%) of biomass (**a**), nitrogen (**b**) and phosphorus (**c**) to different organs in two bamboos in successional fallows.

than those of *D. hamiltonii*. The allocation of potassium, calcium and magnesium showed a pattern similar to biomass allocation and therefore not presented here.

5. Discussion

5.1 *Architectural studies*

Hallé *et al* (1978) established Mc Clure's model to accommodate the basic architecture of bamboos. There are differentiated axes of two kinds: first sigmoid trunk axes which are essentially mixed, originate by basal branching and bear, second, plagiotropic leafy branches; both kinds show determinate growth due to a high degree of preformation. Takemouchi (1931) and Mc Clure (1966), however, differentiated the below-ground and above-ground parts as solid (internodes filled) and hollow (internodes empty) axes respectively. Based on this difference and also based on the fact that the zero-order branch of Hallé *et al* (1978) is in fact a number of branch systems that are sympodially produced, ordering was done, for the present study, with respect to above-ground branches alone, the above-ground main axis being designated as the zero-order branch.

The canopy architecture of *D. hamiltonii* showed plasticity depending upon the fallow age. In a 5 yr old fallow the culm assumes a conical form which is related to more space and light availability. With reduction in space and light in older fallows the culm assumes a cylindrical form. *D. hamiltonii* has limited plasticity with a range of upto 25 yr of the fallow since it is an emergent and shade intolerant species, but optimum growth occurred in 15 and 25 yr old fallows. Partly because of reduced light availability in fallows older than 25 yr and partly because the intermast phase for *D. hamiltonii* is 30–40 yr depending upon the geographical zone (Varmah and Bahadur 1980; Janzen 1976) this species is eliminated during succession after 25 yr (Toky and Ramakrishnan 1983a). Over a successional gradient, *N. dulloa* showed great plasticity extending upto 60 yr old fallow, as an emergent upto 15 yr of fallow regrowth and as an undergrowth in 25 and 60 yr old forests. *N. dulloa* with a reported intermast phase of 15–17 yr (Janzen 1976) is, however, able to regenerate through seeds and survive upto 60 yr of fallow age because of its shade tolerance, though with reduced vigour.

Unlike trees where extension and radial growth are continuous activities throughout the life cycle of the species, growth and elongation in bamboos occur just when the culm is produced. Extension growth is through mere elongation of the zero-order branch, whilst radial growth is through cell enlargement and thickening of preformed tissue. Since successive generations of zero-order branches within a given clum tend to be thicker than the earlier ones (Hallé *et al* 1978) it should be expected that the culms produced in older fallows would be generally thicker, as the stock/regeneration age would increase, though upto a point being constrained by species plasticity. Thus in the larger *D. hamiltonii* maximum diameter for the zero-order branch occurred in a 25 yr old fallow, whereas in the smaller *N. dulloa* it occurred in a 15 yr old fallow.

The extension of the zero-order axis through internodal elongation starts from base upwards with peak elongation occurring during the fifth week as also reported for *Phyllostachus* species (Ueda 1960; Mc Clure 1966). With lesser resource storage in the below-ground organs of *N. dulloa*, sylleptic branch production with leafy shoots on them is helpful for immediate photosynthesis and thus for quicker growth. On the other hand, with more resource availability in the below-ground parts of *D. hamiltonii*, the resources are diverted initially for the growth of the larger zero-order axis with postponement of branch production to the second year. Being light demanders, the two species had a high degree of branch drop-off as light availability decreased at lower canopy positions, a feature which helps in rapid upward shift of the canopy of early successional species (Boojh and Ramakrishnan 1982a; Shukla and Ramakrishnan 1986).

5.2 *Allocation studies*

The biomass of *D. hamiltonii* increased with fallow age upto 25 yr and that of *N. dulloa* declined sharply in fallows older than 15 yr. However, the biomass accumulation rate in both the species peaked in a 15 yr old fallow suggesting that growth conditions are, perhaps, more favourable in this fallow than in others, apart from increased regeneration age of the clump.

During the first 15 yr, considerable fluctuation occurred in the above-ground/below-ground ratio of *N. dulloa*. During the first year of shoot growth, rapid transfer of resources from below-ground to above-ground compartment occurred. Subsequently the photosynthates from the above-ground parts are retranslocated to the below-ground organs resulting in a lower ratio. Subsequently, the shoot growth picked up because of another upward translocation of resources from the below-ground parts resulting in a higher ratio in a 15 yr old fallow. The lowest ratio attained in *N. dulloa* in a 60 yr old fallow may be an adaptation for survival through vegetative growth during the subsequent slash and burn operation. Such a survival strategy was observed in many rhizomatous species such as *Imperata cylindrica* developed in successional fallows after slash and burn agriculture (Saxena and Ramakrishnan 1983) and in *D. hamiltonii* during the present study.

Proportional allocation of phosphorus compared to other nutrients to the below-ground parts was much higher for the two species. A more effective distribution of this element compared to many others for vegetative reproduction was also reported in *Ixia polystachya* (Hocking 1984) and in *Solidago* species (Abrahamson and Caswell 1982). Higher allocation of nitrogen to the leaf component of both the species may be related to photosynthetic efficiency, since leaf nitrogen content is shown to be positively related to photosynthetic rate (Murata 1969; Terry and Ulrich 1973; Nàtr 1975; Field 1983). Such a differential allocation of biomass and nutrients suggest that it is often difficult to establish a connection between fitness and resource allocation based on any one currency, and emphasizes the importance of nutrient allocation in such studies (Van Andel and Verma 1977; Chapin 1980; Williams and Bell 1981; Saxena and Ramakrishnan 1983).

A useful strategy for any species establishing after slash and burn of a forested ecosystem should be to capitalize upon the resources of an enriched substratum, which is transient, and make adequate growth as quickly as possible. The species establishing in such environments could have strategies for establishment from seed or for establishment from vegetative sprouts (Saxena and Ramakrishnan 1983). The bamboo species that are predominantly clonal, with high allocation of resources to the below-ground organs of reproduction (Keeley and Keeley 1977; Gadgil and Prasad 1984), represent an extreme stage among vegetative strategists with seed reproduction occurring once during the life cycle, with very low regeneration (Rao and Ramakrishnan 1987) due to predation and rapid loss in viability and weaker seedlings derived from small seeds.

Thus the growth and architectural patterns of bamboo species are geared to capitalize upon high light regime of an early successional environment to make quick growth before light become limiting, and quickly utilize the nutrient resources of a transient soil environment.

Acknowledgements

The authors are thankful to Prof. Makato Numata of the Chiba University and Prof. Etsuzo Uchimura of Forestry and Forest Products Research Institute, Japan for their helpful comments. This work was carried out at Centre for Eco-Development, North-Eastern Hill University, Shillong and supported by Department of Science and Technology, New Delhi.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 2, April 1988, pp. 135–138.

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Suitable level of zinc, copper and manganese for mass production of the vesicular arbuscular mycorrhizal fungus, *Glomus fasciculatum*

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MS received 29 August 1987; revised 23 January 1988

Abstract. There is no information on the role of micronutrients on the mass production of the vesicular arbuscular mycorrhizal fungi. Hence zinc, copper and manganese at 4 different levels in Ruakura plant nutrient solution viz. ¼, ½ and double the recommended level were studied to select the suitable level for mass production of *Glomus fasciculatum* inoculum raised in *Chloris gayana* (Rhodes grass) using perlite: soilrite mix (1 : 1 by volume). Percentage root colonization, extramatrical chlamydospore number and number of infective propagules per unit weight of the inoculum were maximum at half the recommended level i.e. 0·18, 0·04, 0·73 mM of Zn, Cu and Mn respectively.

Keywords. *Glomus fasciculatum*; VA mycorrhiza; mass production; zinc; copper; manganese; perlite: soilrite mix; Rhodes grass; Ruakura nutrient solution.

1. Introduction

Vesicular arbuscular mycorrhizal (VAM) fungi have not been cultured on artificial nutrient medium. The obligate symbiotic nature of VAM presently dictates that all VAM inoculum must be grown on roots of an appropriate host in pot cultures (Wood 1984; Sreenivasa and Bagyaraj 1988). When partially artificial substrates are used to raise the pot cultures of VAM (Sreenivasa 1986) addition of a plant nutrient solution is required for optimum plant growth. Ruakura plant nutrient solution which has both major and minor elements, after comparison with different nutrient solutions, has been recommended as best in improving plant growth (Smith *et al* 1983).

Micronutrients such as Zn, Cu and Mn can be toxic to VAM fungi at higher concentration and are known to inhibit the spore germination (Hepper 1979; Gildon and Tinker 1983). However, zinc at low concentration was stimulatory to spore germination (Mcllveen and Cole 1978). There is no information regarding the optimum level of Zn, Cu and Mn needed to produce maximum infective propagules of VAM in commercial pot cultures. Hence the present study was undertaken to find out the optimum level of Zn, Cu and Mn for mass production of the VAM fungus, *Glomus fasciculatum.*

2. Materials and methods

Earlier studies conducted in this laboratory demonstrated that Rhodes grass (*Chloris gayana* Kunth) is the best host (Sreenivasa and Bagyaraj 1988) and perlite: soilrite mix (1 : 1 by volume) is the best substrate (Sreenivasa 1986), for mass production of *G. fasciculatum.* Hence, this host and substrate were used in the present study. Soilrite used in the substrate mix is a commercial preparation with a horticultural grade expanded perlite, exfoliated vermiculite and peat moss in the proportion of $\frac{1}{3}:\frac{1}{3}:\frac{1}{3}$ by volume. The substrate mix used in the study had a pH of 6·4 and 0·13, 0·03 and 0·16% total N, P and K respectively. Ruakura plant nutrient solution (Smith *et al* 1983) was modified by changing the levels of Zn, Cu and Mn to $\frac{1}{4}$, $\frac{1}{2}$ and double the recommended level and compared with the unamended solution. The recommended levels of Zn, Cu and Mn in the Ruakura nutrient solution are 0·36, 0·08 and 1·46 mM (i.e. 24, 5 and 80 ppm) respectively. When the level of one element in the nutrient solution was changed, all other elements were added at the recommended level. The sources of Zn, Cu and Mn in the Ruakura nutrient solution were $ZnCl_2{\cdot}2H_2O$, $CuCl_2{\cdot}2H_2O$ and $MnCl_2{\cdot}4H_2O$ respectively. Perlite: soilrite mix (1:1 by volume) was filled to 16 cm diameter earthen pots of 3 kg capacity. Pots were inoculated by placing a thin layer of *G. fasciculatum* (Thaxt.) Gerd and Trappe inoculum (30 g/pot having 2100 infective propagules/g as determined by MPN assay, Porter 1979) 2 cm below the substrate surface. Rhodes grass seeds were sown and covered with a thin layer of the substrate. Pot cultures were maintained in a glass house with a mean temperature range of 28–32°C. Four replications with uniform plant population were maintained in each treatment and watered whenever necessary. Modified Ruakura nutrient solution with different levels of Zn, Cu and Mn were given to pots (50 ml/pot) on days 10, 30 and 45 after sowing.

The crop was harvested on 60th day and plant dry weights were recorded. Per cent root colonization was determined by staining the roots with trypan blue (Phillips and Hayman 1970) and the spore count by wet sieving and decanting technique (Gerdemann and Nicolson 1963). The number of infective propagules in the pot ball (total pot content), after chopping the roots to 1 cm bits, was determined by MPN technique, using onion as the test plant (Porter 1979). The soil dilutions used in the bioassay ranged from 10^{-5}–10^{-9}. After growing onion seedlings for 6 weeks, the roots were examined for mycorrhizal colonization. The most probable number of infective propagules was computed referring to MPN table given by Alexander (1965). The total Zn, Cu and Mn content of the substrate was determined after HCl digestion. Using suitable dilutions of the digested extract absorbance at 215, 325 and 280 nm was read in an atomic absorption spectrophotometer to know the amount of Zn, Cu and Mn respectively (Black 1965).

3. Results and discussion

Percentage mycorrhizal root colonization, extramatrical chlamydospore number and number of infective propagules per unit weight of the inoculum as influenced by the different levels of Zn, Cu and Mn are given in tables 1–3. All the mycorrhizal parameters studied were highest at half the recommended level of 3 micronutrients in the nutrient solution i.e. 0·18, 0·04 and 0·73 mM of Zn, Cu and Mn respectively and least at double the recommended level. The mycorrhizal parameters in pots receiving recommended level of Zn, Cu and Mn were significantly less compared to pots receiving half the recommended level. This is perhaps due to the presence of these micronutrients in the substrate mix, perlite and soilrite (table 4). At double the recommended level, the micronutrient concentration might have reached the level inhibitory to VAM fungi. Gildon and Tinker (1983) observed that Zn at 1·15 mM and Cu at 0·39 mM inhibited mycorrhizal colonization of onion plants by *G. mosseae*. Similar observations were also made by McIlveen and Cole (1978) in

Table 1. Effect of different levels on Zn on per cent root colonization, spore number and infective propagules of *G. fasciculatum* inoculum, and shoot and root dry biomass of Rhodes grass.

Level of Zn (mM)	Root colonization (%)	Spore number/ 50 ml substrate	Number of infective propagules/g ($\times 10^7$)	Shoot dry biomass (g/plant)	Root dry biomass (g/plant)
0·09	76(61)	397	0·39	4·85	2·31
0·18	94(76)	494	0·79	9·39	4·18
0·36	84(66)	449	0·61	9·48	4·65
0·72	70(56)	322	0·16	9·89	4·78
F test significant at $P=0{\cdot}05$	—	—	—	—	—
LSD at $P=0{\cdot}05$	6·0	27·3	—	0·18	0·27

Per cent root colonization values after arcsin transformation are given in parantheses.

Table 2. Effect of different levels of Cu on per cent root colonization, spore number and infective propagules of *G. fasciculatum* inoculum, and shoot and root dry biomass of Rhodes grass.

Level of Cu (mM)	Root colonization (%)	Spore number/ 50 ml substrate	Number of infective propagules/g ($\times 10^7$)	Shoot dry biomass (g/plant)	Root dry biomass (g/plant)
0·02	74(60)	368	0·32	4·72	2·37
0·04	94(76)	509	0·91	9·50	4·26
0·08	84(66)	443	0·58	9·74	4·30
0·16	71(57)	323	0·16	9·85	4·96
F test significant at $P=0{\cdot}05$	—	—	—	—	—
LSD at $P=0{\cdot}05$	8·2	35·9	—	0·41	0·26

Per cent root colonization values after arcsin transformation are given in parentheses.

Table 3. Effect of different levels of Mn on per cent root colonization, spore number and infective propagules of *G. fasciculatum* inoculum, and shoot and root dry biomass of Rhodes grass.

Level of Mn (mM)	Root colonization (%)	Spore number/ 50 ml substrate	Number of infective propagules/g ($\times 10^7$)	Shoot dry biomass (g/plant)	Root dry biomass (g/plant)
0·37	72(58)	406	0·50	4·19	2·15
0·73	94(76)	503	0·85	9·19	4·08
1·46	85(67)	429	0·53	9·73	4·18
2·92	65(53)	335	0·20	9·81	4·22
F test significant at $P=0{\cdot}05$	—	—	—	—	—
LSD at $P=0{\cdot}05$	5·4	31·3	—	0·46	0·26

Per cent root colonization values after arcsin transformation are given in parentheses.

Table 4. Total zinc, copper and manganese content of the substrate used in the study.

	Zn (μg/g)	Cu (μg/g)	Mn (μg/g)
Perlite	1·05	0·97	4·82
Soilrite	15·00	2·74	43·19
Perlite : soilrite mix (1 : 1 by volume)	5·79	1·47	17·86

soybean—*G. mosseae* symbiosis. Zn, Cu and Mn applied at half the recommended level also significantly increased the shoot and root biomass compared to these elements applied at one fourth of the recommended level. The difference in the shoot and root biomass between plants given half the recommended level, the recommended level and double the recommended level were not generally significant statistically, except one or two exceptions. In these 3 levels, there was no correlation between the root biomass and the number of infective propagules per unit weight of the inoculum. The results of the present study clearly indicates that Zn, Cu and Mn can be used at half the recommended level in Ruakura nutrient solution for the mass multiplication of *G. fasciculatum.*

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 2, April 1988, pp. 139–148.
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Cytology and distribution pattern of woody species of Verbenaceae in Palni hills

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MS received 1 June 1987; revised 3 March 1988

Abstract. Studies on the cytology and distribution pattern of 16 woody species of Verbenaceae from Palni hills have shown that: (i) in case of *Lantana*, the chromosome size is not influenced by increase or decrease in chromosome numbers, (ii) there is no correlation of chromosome size with the habits of different species of *Vitex*, (iii) transmigration of chromatin material in *Gmelina arborea*, *Lantana aculeata* and *Symphorema polyandrum* results in encountering of abnormal chromosome numbers, (iv) in *Lantana camara* loose or compact association of various groups of chromosomes indicates that genomic constitution of the species is made up of intermingling of different genomes, (v) *L. camara* (4x) and *L. indica* (6x) do show the presence of multiple associations in some pollen mother cells which indicate their autoalloploid nature, and (vi) in *Nyctanthes arbortristis*, the correct recorded number is 18 bivalents and the earlier reports of n = 22 and n = 24 cannot be explained easily.

The distribution pattern of various species in Palni hills as worked out here shows that in case of the *Lantana*, tetraploid species have wider range of distribution as compared to 2x and 6x ones. The maximum representation of the presently studied species is between 900–1400 m altitudinal zone illustrating that each species is represented in more than one type of forests while overlapping of distribution in different forests is not a rare phenomenon of the investigated taxa.

Keywords. Chromosome numbers; distribution; polyploidy; Verbenaceae.

1. Introduction

Family Verbenaceae is one of the largest families of flowering plants and is widely dispersed in nearly all warm and temperate regions with about 78 genera and 3,000 species. It is of considerable economic importance in providing timber yielding, medicinal and ornamental plants. A perusal of cumulative information on chromosome numbers of the family indicate that members from tropical regions, by and large, remain uninvestigated. One such region is comprised of south Indian forests from where only isolated chromosome reports are available to-date and there is no consolidated work. To fill in this gap, the present study was carried out on the woody members of the family from Palni hills (Tamil Nadu).

2. Material and methods

Young flower buds of wild plants growing in different forests of Palni hills were fixed in Carnoy's fluid (6 parts ethanol: 3 parts chloroform: 1 part glacial acetic acid saturated with iron acetate) for 24 h and then transferred to 95% ethanol. Usual acetocarmine smears were employed for chromosomal analysis. Photomicrographs were taken from permanent slides mounted in euparal after following usual dehydrating procedure. Pollen grains were observed by staining with 50% glycero-

Table 1. Chromosome numbers in certain members of Verbenaceae from Palni hills.

Taxa	Localities (PUN Accession Numbers*)	Recorded chromosome number and ploidy levels	Remarks
1. *Clerodendron infortunatum* Linn	Sabri Kadu 1,300 m (28656)	n = 26 = 4x	Gajapathy (1962) recorded n = 24, Bir and Saggoo (1979) n = 26
2. *C. serratum* spr.	Palamalai 1,200 m (26812)	n = 26 = 4x	Earlier Bir and Kumari (1979) recorded n = 24 from Pachmarhi hills
3. *Gmelina arborea* Roxb.	Perumalmalai 1,600 m (26726)	n = 20 = 2x	Raman and Kesavan (1963) recorded n = 19 from eastern India, Mehra and Bawa (1969) from eastern Himalaya and Gill *et al* (1983) from Pachmarhi hill also noted the same number
4. *Holmskioldia sanguinea* Retz.	Perumalmalai, 1,600 m	n = 16 = 2x	Malla *et al* (1977) recorded n = 18
5. *Lantana aculeata* Linn.	Uttu 1,200 m (26840) Tiger shola, 1800 m (28672)	n = 22 = 4x	—
6. *L. camara* Linn.	Perumalmalai 1,600 m (26750) Sabri Kadu 1,500 m (26824)	2n = 44 = 4x	Earlier n = 11, 33, 2n = 33 have been reported by Natarajan and Ahuja (1957)
7. *L. indica* Roxb.	Sabri Kadu 1,500 m (26804) Sabri Kadu 1,400 m (26805)	2n = 66 = 6x	Malik and Ahmed (1963) recorded n = 11 from Pakistan and from India by Natarajan and Ahuja (1957)
8. *L. montevidens* Brig.	Dolman's Circle 1,400 m	n = 11 = 2x	Earlier n = 18 recorded by Raghavan and Arora (1960)
9. *L. wightiana* Wall.	Palamalai 1,260 m (28418)	n = 22 = 4x	Arora (1960) recorded 2n = 72
10. *Nyctanthes arbortristis* Linn.	Palni 250 m (26832)	n = 18 = 2x	Previously 2n = 44 is recorded by Nanda (1962), Sharma and Mukhopadhyay (1963) and Mehra (1976) and 2n = 24 by Kundu and De (1968)
11. *Premna tomentosa* Willd.	Periya Shola forest 1,800 m (26774)	n = 19 = 2x	—
12. *Symphorema involucratum* Roxb.	Periya shola (26742) 1,000 m	n = 14 = 2x	—
13. *S. polyandrum* Wt.	Sabri Kadu 1,200 m (28588)	n = 14 = 2x	—
14. *Vitex altissima* Linn. (= *V. alata* Heyne)	Uttu, 1,100 m (26846)	n = 12 = 2x	—
15. *V. arborea* Roxb. (= *V. pubescens* Vahl.)	Fall's view 900 m (26698)	n = 12 = 2x	—
16. *V. negundo* Linn.	Kodaikanal road 300 m (26720)	n = 13 = 2x	Earlier Malik and Ahmed (1963) recorded n = 12, Sobti and Singh (1961); n = 13, Mehra and Bawa (1969); n = 16, Sharma and Mukhopadhyay (1963) and Mehra (1976) n = 17 from different parts of India

*Based on cumulative chromosomal data by Fedorov (1969); Love and Love (1961); Cytotaxonomical Atlas of Arctic Flora (1975); Index to Chromosome Numbers 1956 onwards; IOPB Chromosome Number Reports I-LXXXXI; SOCGI Chromosome Number Reports I-III.

*These are the representative localities where the taxa grow in maximum abundance otherwise for population analysis materials were examined from different areas of distributional range (see figure 1).

acetocarmine. Uniformly stained and well filled pollen were scored as fertile while wrinkled and/or unstained pollen were taken as sterile ones. Vouchers are preserved in the Herbarium of the University, PUN numbers are mentioned in parentheses after localities indicated in table 1.

3. Observations

3.1 *Cytological analysis*

Chromosomal information about species analysed is given in table 1. Meiosis is perfectly normal in *Clerodendron infortunatum* n = 26, *C. serratum* n = 26, *Holmskioldia sanguinea* n = 16, *Lantana montevidens* n = 11, *Nyctanthes arbortristis* n = 18, *Symphorema involucrata* n = 14, *Premna tomentosa*, n = 19, *Vitex altissima* n = 12 (figure 18), *V. arborea* n = 12 (figure 19) and *V. negundo* n = 13 resulting into normal microsporogenesis and almost cent per cent pollen fertility except in *C. serratum* (91·4%), *P. tomentosa* (94·4%), *V. negundo* (93·4%) and *V. altissima* (92·4%) while in *Gmelina arborea* (86·8%), *Lantana aculeata* (26·4%), *L. camara* (58·0%), *L. indica* (56·0%), and *Symphorema polyandrum* (93·4%) and abnormal microsporogenesis leads to certain amount of pollen sterility. Main points of interest are as follows:

(i) In case of *L. camara* n = 22 and *L. indica* n = 33 normal bivalents formation is noticeable in a large number of pollen mother cells (PMCs) but the presence of multivalents and univalents is not a rare phenomenon. In case of *L. camara* the frequency of multivalents per PMC varies from $0\text{–}1_{\mathrm{V}}$, $1\text{–}4_{\mathrm{IV}}$, $0\text{–}3_{\mathrm{III}}$, the number of IIs varies from 11–19 and of univalents from 1–6 (figures 3, 6). On the basis of analysis of chromosomal configurations, the average frequency per PMC is $0{\cdot}44_{\mathrm{V}}$, $2{\cdot}55_{\mathrm{IV}}$, $0{\cdot}94_{\mathrm{III}}$, $13{\cdot}38_{\mathrm{II}}$, $3{\cdot}05_{\mathrm{I}}$ giving 2n = 44. Multiple associations account for 30·80% of the chromosomes while bivalents and univalents share 60·81 and 6·93% chromosomes respectively. Laggards are frequently encountered at A-I and A-II. One to two chromatin bridges are also common at A-I (figures 4, 5). These may be due to interlocking of chiasmata. All this leads to abnormal microsporogenesis with only 58% fertile pollen.

(ii) In *L. indica*, in 41·67% of the examined PMCs at diakinesis and M-I, 33 bivalents are clearly constituted (figures 15, 16) whereas in rest of the 58·33% of the PMCs at diakinesis and M-I besides bivalents, multivalents and univalents have been observed. The number of V, IV, III, II and I range from 0–1, 0–3, 0–2, 20–33 and 0–9 respectively with average frequency of chromosomal associations for PMC being $0{\cdot}268_{\mathrm{V}}$, $1{\cdot}08_{\mathrm{IV}}$, $0{\cdot}70_{\mathrm{III}}$, $26{\cdot}79_{\mathrm{II}}$ and $3{\cdot}79_{\mathrm{I}}$. Bivalents and multivalents account for 81·16 and 11·72% of involved chromosomes respectively. Generally 1–4 laggards were seen at T-II. All this leads to abnormal microsporogenesis with only 56% pollen fertility. Often micropollen are noticeable. It is interesting to note that seed setting is quite low in this species as particularly noticed in individuals from Perumalmalai (1,600 m) and Dolmen's circle (1,400 m).

(iii) Transmigration of chromatin material has been observed in *G. arborea* n = 20, *L. aculeata* n = 22 (figure 9) and *S. polyandrum* n = 14 (figure 17) involving different number of PMCs. This results in encountering of normal chromosome numbers (figures 12, 13). Cytoplasmic channels were found to be restricted at M-I in case of

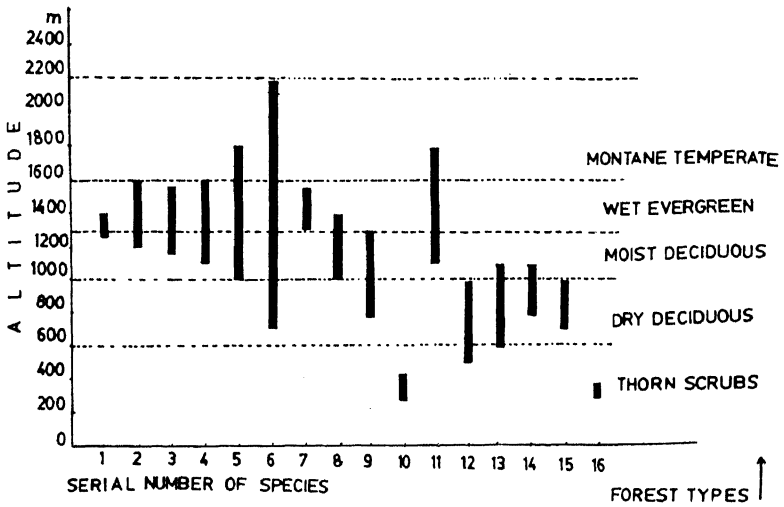

Figure 1. Distribution patterns of various species in Palni hill forests of south India. The serial number of the species are the same as in table 1.

G. arborea and *S. polyandrum* as interconnected PMCs were not evident at the later stages of meiosis. However, in case of *L. aculeata*, cytoplasmic channels were observed at later stages of meiosis. As a result of this, microsporogenesis is highly abnormal and pollen fertility is greatly reduced. Small, medium and large sized pollen were indiscriminately fixed up.

(iv) An interesting feature of *L. camara* from Sabri Kadu 1,500 m, is that, quite often loose or a compact associations of various groupings of bivalents are evident (figures 7, 8, 10, 11). Generally 6 such quadrivalents (loose associations of two bivalents) are seen. In majority of the observable PMCs other types of associations were not lacking (table 2). A close study of associated bivalents indicated that association sites are not specific. Different types of associates indicate that the genomic constitution of *L. camara* is made up through intermingling of different genomes and thus it is a hybrid species. However, the course of meiosis is normal. The pollen fertility is reduced to 87%.

(v) Majority of the studied taxa have medium sized chromosomes. The small sized chromosomes are seen in *C. infortunatum* (figure 2) and the largest size in case of *S. involucrata*. In case of 5 species of *Lantana* (all shrubby) it is pertinent to point out that the chromosome size is not influenced by the increase or decrease in chromosome number and all these have medium sized chromosomes. The chromosome size also does not show any correlation with habit of different species of *Vitex*, the 2 species *V. altissima* (average chromosome size $2{\cdot}21 \pm 1{\cdot}48\ \mu m$) and *V. arborea* (average chromosome size $2{\cdot}14 \pm 1{\cdot}46\ \mu m$) do not show any marked chromosome size differences from the shrubby species of *V. negundo* (average chromosome size $2{\cdot}14 \pm 1{\cdot}46\ \mu m$).

(vi) *N. arbortristis* shows regular constitution of 18 bivalents at M-I (figure 14) and 18 : 18 chromosomal distribution at A-I. Earlier, recorded numbers for the species are n = 22 and n = 24 (table 1). The meiosis is normal and pollen are nearly 100% stainable.

3.2 *Distribution pattern*

In Palni hills the distribution pattern of various investigated taxa of Verbenaceae is shown in figure 1 (also see table 1). Tetraploid plants of *Lantana* are well adapted in various forests. These proliferate very quickly in all types of habitats. An analysis of a large number of populations clearly exhibited that the tetraploid *Lantana* species have wider altitudinal distribution and can colonize variable habitats while the diploid and hexaploid species are very rare and are restricted in distribution (see also Stebbins 1971). According to Natarajan and Ahuja (1957) the wide spread occurrence of *Lantana* in India may be due to polyploidization and adaptations of plants for apomictic propagation which may be responsible for maintaining even sterile types like 3x and 5x.

Vitex species grow in the moist deciduous (900 m) and evergreen regions (1,100 m) of the Palni hills and the trees are absent at lower (below 700 m) as well as higher altitudes (above 1100 m). Trees of *V. arborea* studied from different regions at an altitude of 900–1100 m do not show any phenotypic variations when their characters as leaf size, shape, texture and veins, length of petiole, tree height, bole characters, crowns, flower size, number of flowers per panicle, etc are taken into consideration. Similar is the case with the individuals of *V. altissima*. *Strebulus* is confined to dry deciduous/moist deciduous forests of low hills, only towards Periyakulum forests. *Clerodendron* species are only found in the upper hills along the margin of shola forests and do not show phenotypic variability. *G. arborea* has two biotypes, one with larger leaves (17–23 × 13–15 cm i.e. length × breadth lamina, petiole 1·7–2 cm) found in evergreen forests of Perumalmalai and second with smaller leaves (13–18 × 6·5–10 cm i.e. length × breadth lamina, petiole 3·8–4·2 cm) growing along the margins of dry deciduous forests of Palamalai. This tree lends a distinct physiogonomy to evergreen forests because of its deciduous nature. *Symphorema* is a woody climber available along margin of an intermediate dry as well as moist deciduous forests (near Fall's view) but absent in deep forests of dry and moist deciduous nature. Similarly, *Premna* is also restricted to lower dry hills. All these taxa, by and large, have broad adaptive significance to various types of forests (figure 1) but wherever their distribution range is wider, they flourish the maximum in the middle of the range and number of individuals decreases towards the extremes/outer limits. There is normal seed setting of these trees/shrubs but due to lack of special dispersal devices, the seeds mostly remain confined to specific forests and that is the reason for restricted distribution particularly in case of *Premna*, *Strebulus* and *Symphorema*.

4. Discussion

4.1 *Chromosome numbers**

Clerodendron is an interesting genus in that it is based on $x = 12$–15 and 23 since its various species show $n = 12$, 15, 21, 23, 24, 25, 26, 30, 46, 54 and 92. These include both aneuploid types as well as intraspecific polyploid series. For this genus, Choudhary and Roy (1983) are of the opinion that chromosome numbers $n = 21$, 23, 24, 25 have arisen from a common stock of $x = 12$. *Gmelina* ($x = 18$, 19, 20) and

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*See foot note to table 1.

Nyctanthes (x = 18, 22, 24) are tribasic and *Holmskioldia* is dibasic (x = 16, 22) while *Symphorema* (x = 14) and *Premna* (x = 19) are monobasic. *Lantana* has also series of chromosome number with x = 11, 15, 18, 19 but x = 11, 18 are the most common numbers. It is surprising to note that species based on x = 11 form polyploid series

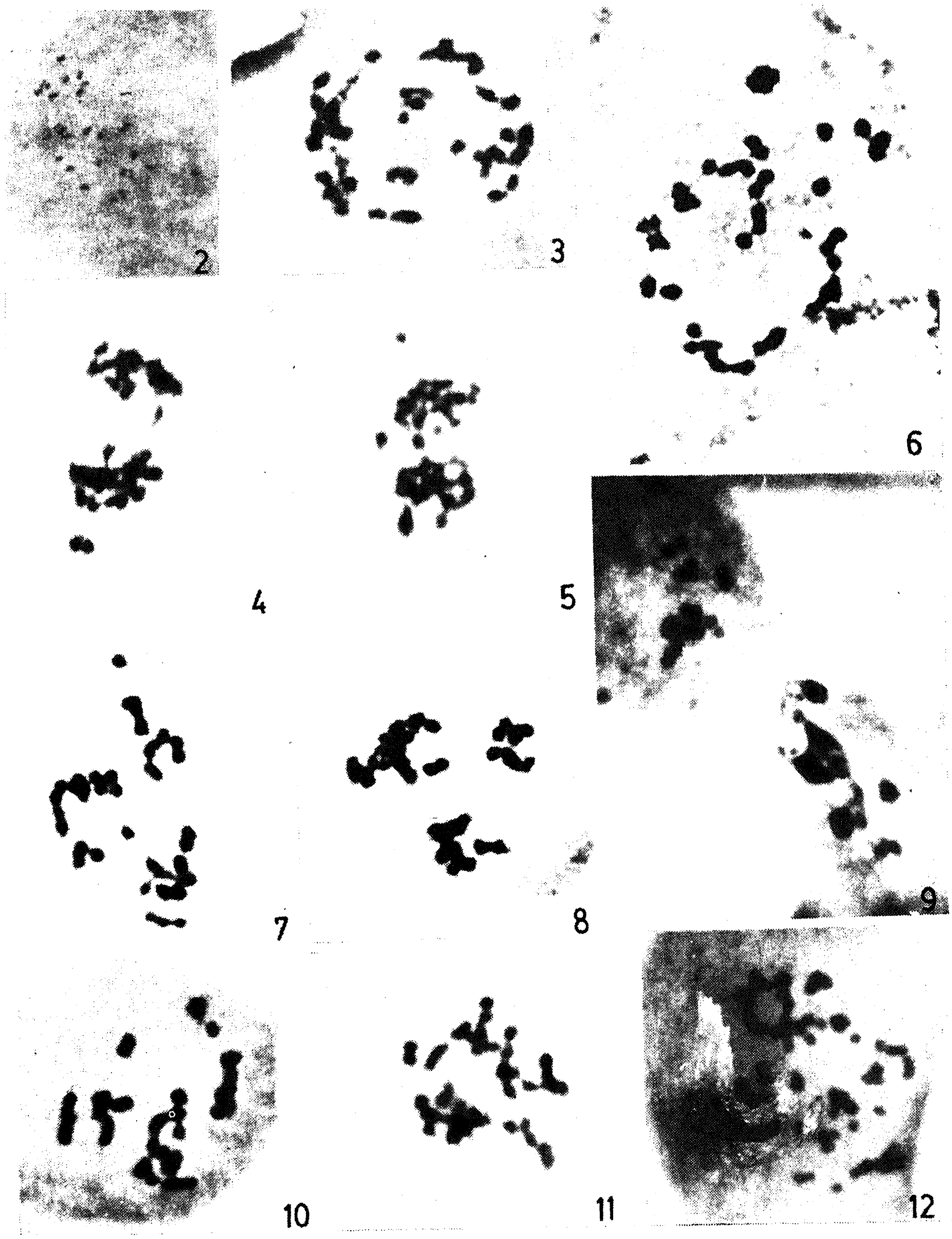
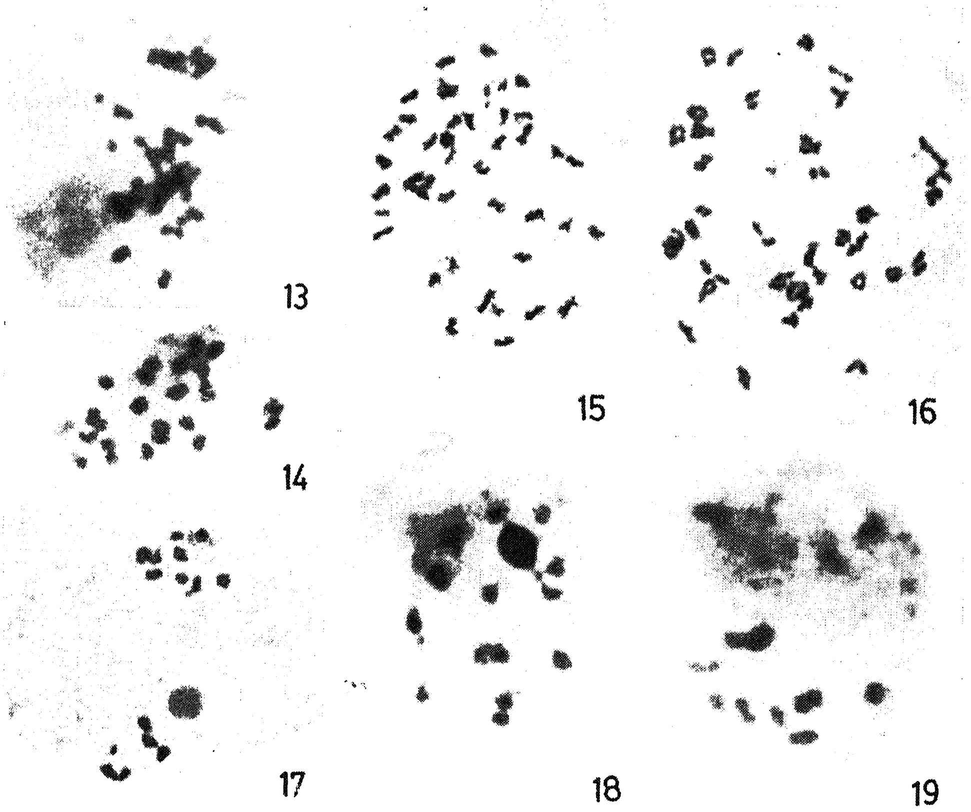


Figure 2–12. For caption, see p. 145.



Figures 2–19. Meiosis in Verbenaceae (×1800). **2.** *C. infortunatum*, M-I showing n = 26. **3–11.** *L. camara*. **3.** Diakinesis with 22_{II}. **4–5.** Chromatin bridges at A-I. **6.** Multivalents and univalents at M-1. **7–8, 10–11.** Secondary associations at M-I. **7.** Association of 1(6)+1(5)+1(3)+4(2). **8.** Association of 13(1)+3(7)+2(2)+2(1). **10.** Association of 8(1)+4(1)+3(2)+4(1). **11.** Association of 2(5)+3(2)+6(1). **9–12, 13.** *L. aculeata*. **9.** PMCs showing cytomixis. **12–13.** Gain in chromosomes showing pseudomultivalents, bivalents, univalents and fragments. **14.** *N. arbortristis*, M-I with 18_{II}. **15–16.** *L. indica*, 33 bivalents at diakinesis. **17.** *S. polyandrum*, Diakinesis showing 14_{II}. **18.** *V. altissima*, diakinesis showing 12_{II}. **19.** *V. arborea*, M-I with 12_{II}.

whereas those with x = 18 show aneuploid numbers. Maybe, the members of the groups based on x = 11 and x = 18 have evolved from different stocks or the common stock from which these diverged, has become obliterated. Lewis (1967) has termed such types of chromosomal evolution as 'cytocatalytic evolution'. *Vitex* has chromosome number n = 12, 13, 16, 17 with abundance of inter- and intraspecific aneuploidy. The incidence of aneuploid numbers in *Vitex* does not upset the regular bivalent formation. Discussing the incidence of aneuploid numbers in *Clerodendron* (x = 12, 15), Sharma and Mukhopadhyay (1963) inferred that regular bivalent formation in aneuploid normal species suggest that the resultant evolution through several generation has ultimately saturated to structural changes in the duplicated chromosomes and then multivalents are eliminated. Amongst the basic numbers for the genus, x = 16 is the most common.

4.2 *Chromosome size*

The size of chromosomes cannot be correlated with either n-number or the ploidy level. It is a reflection of phyletic lineage or manifestation of evolutionary mechanisms. For example, *Lantana* shows different evolutionary lines. There is much evolution of species through apomixis, clonal propagation, hybridization and polyploidy (Natarajan and Ahuja 1957). The members with medium sized chromosomes in *Lantana* show marked adaptability in the nature, spreading in different altitudinal variations in Palni hills.

The presence of multivalents ($\mathrm{V_s}$, $\mathrm{IV_s}$, $\mathrm{III_s}$) in certain individuals of *L. camara* and *L. indica* would point to the autoalloploid nature of their origin. The total presence of bivalents in some of the individuals shows the stabilization of such species in nature. As noted earlier, the medium sized chromosomes in *L. camara* and *L. indica* show both the formation of bivalents only or the formation of $\mathrm{V_s}$, $\mathrm{IV_s}$, $\mathrm{III_s}$ etc. On one side according to Burnham (1956) the smaller chromosomes are less prone to multivalent formation and on the other side, Morrison and Rajhathej (1980) have clearly shown that multivalents are formed irrespective of chromosome size. All this signifies that bivalents or multivalents formation is not governed by chromosome size.

4.3 *Secondary associations*

Such associations have been recorded for the first time in *L. camara*. As per details in table 2, the chromosomal associations are of very complex nature. The phenomenon is explainable for the fact that certain specific sites of the chromosomes have similar loci on the other chromosomes which join to their close associates. Depending upon the associations between large number of chromosomes it is clear that almost all of these have similar joining sites (table 2). It is evident that *L. camara* is a complex species which contains genomes from its close relatives by hybridization as well as diplodization. The common pattern of secondary association is 2(5) + 3(2) + 6(1).

Table 2. Secondary chromosomal associations at diakinesis/M-I in *L. camara* (2n = 44, 4x).

Number of PMCs observed	Groups of various chromosomal association	Maximum probability	Common
2	1(4) + 2(2) + 14(1)	17	
1	6(2) + 10(1)	16	
2	2(4) + 2(2) + 10(1)	14	
1	4(3) + 2(2) + 6(1)	12	
1	10(2) + 2(1)	12	
2	3(4) + 2(3) + 4(1)	9	
1	2(4) + 2(3) + 3(2) + 2(1)	9	
2	1(8) + 1(6) + 2(2) + 4(1)	8	
2	1(6) + 1(5) + 1(3) + 4(2)	7	
2	1(8) + 1(6) + 1(4) + 4(1)	7	
3	3(4) + 2(2) + 6(1)	11	
5	2(5) + 3(2) + 6(1)	11	2(5) + 3(2) + 6(1)
4	1(6) + 1(5) + 1(3) + 8(1)	11	
3	1(8) + 1(4) + 1(2) + 8(1)	11	
3	2(5) + 1(4) + 8(1)	11	
Total 34			

The various types of secondary groupings of chromosomes (table 2) indicate that chromosomes of these groups have certain amount of homology (Agarwal 1983).

4.4 *Polyploidy*

Polyploidy at 4x, 8x, 9x is frequently encountered in *Clerodendron* (incidence is 88·3%). *Lantana* has 60% taxa at various ploidy levels (3x, 4x, 6x, 8x). Polyploidy is altogether absent in the rest of the investigated genera. High basic chromosome numbers is *Gmelina*, *Nyctanthes*, *Holmskioldia* and *Vitex* seem to be of palaeoploid origin (Lewis 1980). These seem to have evolved from basic stocks with $x=11$, 12 and 13 with loss or gain of chromosomes in due course of evolution in polyploid series.

4.5 *Phylogeny*

On the basis of morphological similarities of *N. arbortristis* with Oleaceae and the incidence of $x=23$ in that family, Kundu and De (1981) suggested that the genus should be placed in a distinct family but near Oleaceae. On the other hand, Sharma and Mukhopadhyay (1963) on the basis of karyotypic similarities suggested that the genus should be placed in Verbenaceae but in a new tribe Nyctanthioideae between Callicarpaceae and Tectoneae. This supported the view of Airy Shaw (1973). On the basis of morphological, anatomical, cytological and embryological feature, Kiew and Baas (1984) found *Nyctanthes* to be a more compatible genus in Oleaceae tribe Jasmineae rather than Verbenaceae. They argued that the treatment of the genus in the Verbenaceae or as a separate family does not reflect its true affinities. If chromosome numbers can be given any weightage then the relationship of *Nyctanthes* with Verbenaceae is more certain.

Acknowledgements

The authors are thankful to the University and Council of Scientific and Industrial Research, New Delhi for financial assistance for the work on the 'Cytogenetics of woody taxa of south India'.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 2, April 1988, pp. 149–156.
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Development of gametophytes, embryogeny and pericarp in *Goodyera repens* (Orchidaceae, Neottieae)

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MS received 4 May 1987; revised 25 January 1988

Abstract. The gametophytes, embryogeny and pericarp of the orchid *Goodyera repens* are described. The anther wall consists of an epidermis, fibrous endothecium, middle layer and a uniseriate tapetum of binucleate cells. Meiosis is normal. Decussate, isobilateral, linear and tetrahedral pollen tetrads are formed. At shedding the massulae are 2-celled. Ovules are anatropous, bitegmic and tenuinucellate. The inner integument alone forms the micropyle. Development of the female gametophyte is monosporic. The primary endosperm nucleus degenerates without any division. Development of embryo corresponds to the Asterad type. The mature embryo lacks differentiation. The seeds are minute and nonendospermic. The seed coat is formed entirely by the outer layer of testa.

Keywords. *Goodyera repens*; gametophyte; integuments initiation; embryo; pericarp; Orchidaceae.

1. Introduction

Earlier works on the embryology of orchids have been reviewed by Schnarf (1931), Swamy (1949a, b), Davis (1966) and Wirth and Withner (1959). Details on the embryology of the genus *Goodyera* however, are fragmentary and restricted to a few species. Afzélius (1916) has given account of megasporogenesis in *G. repens*, while Treub (1879) and Leavitt (1901) had made some cursory observations on the nature of the suspensor of *G. discolor*, *G. pubescens* and *G. tesselata*. As there is no detailed account of the gametophytes, embryogeny and pericarp of any species of *Goodyera*, the present investigation was undertaken.

2. Materials and methods

Buds and flowers of the plant were collected from Shimla hills. Killing and fixation of the material were done using formalin-acetic acid-alcohol. Dehydration, infiltration and embedding were done in the conventional way. Sections were cut 7–12 μm thick and stained with safranin-fast green. Camera lucida drawings were made at the bench level.

3. Results

3.1 *Microsporangium, microsporogenesis and male gametophyte*

The anthers are quadrilocular as seen in the transection. In a very young anther the microspore mother cells are surrounded by an epidermis, an outer secondary parietal layer (*osp*) and an inner layer (*isp*) (figure 1). Periclinal divisions in the *isp* result in two daughter cell layers, of which the outer forms the middle layer and the inner differentiates into the tapetum (figures 1, 2). The *osp* forms the endothecium. The development of the anther corresponds to the Monocotyledonous type. The tapetal cells are initially uninucleate (figure 1) but become binucleate in the later stages (figures 2, 5). The tapetum is of the glandular type. At maturity the anther wall comprises of an epidermis and endothecium showing fibrous thickenings (figure 4). The cells of the middle layer and tapetum degenerate (figure 3).

The microspore mother cells undergo meiotic divisions (figures 6–9) resulting in tetrahedral, decussate, linear and isobilateral microspore tetrads (figures 10–13) forming massulae (figure 4). The microspore nucleus divides to form a large vegetative and a small generative cell. At shedding stage, the spores of the massulae are 2-celled (figure 4).

3.2 *Megasporangium*

At anthesis, numerous ovular primordia develop from the placental ridges (figure 40), each of which consists of an axial row of 7–9 cells surrounded by an epidermal layer (figure 14). At the archesporial cell stage, the inner integument differentiates in the epidermal layer of the ovular primordium (figures 15–19) and is two cells thick (figures 15–18). The micropyle is formed by the inner integument (figure 20). The outer integument primordium (figure 16) differentiates in the same way as that of the inner one (figures 16–18) but grows beyond the inner integument after fertilization. The mature ovules are anatropous, bitegminal and tenuinucellar.

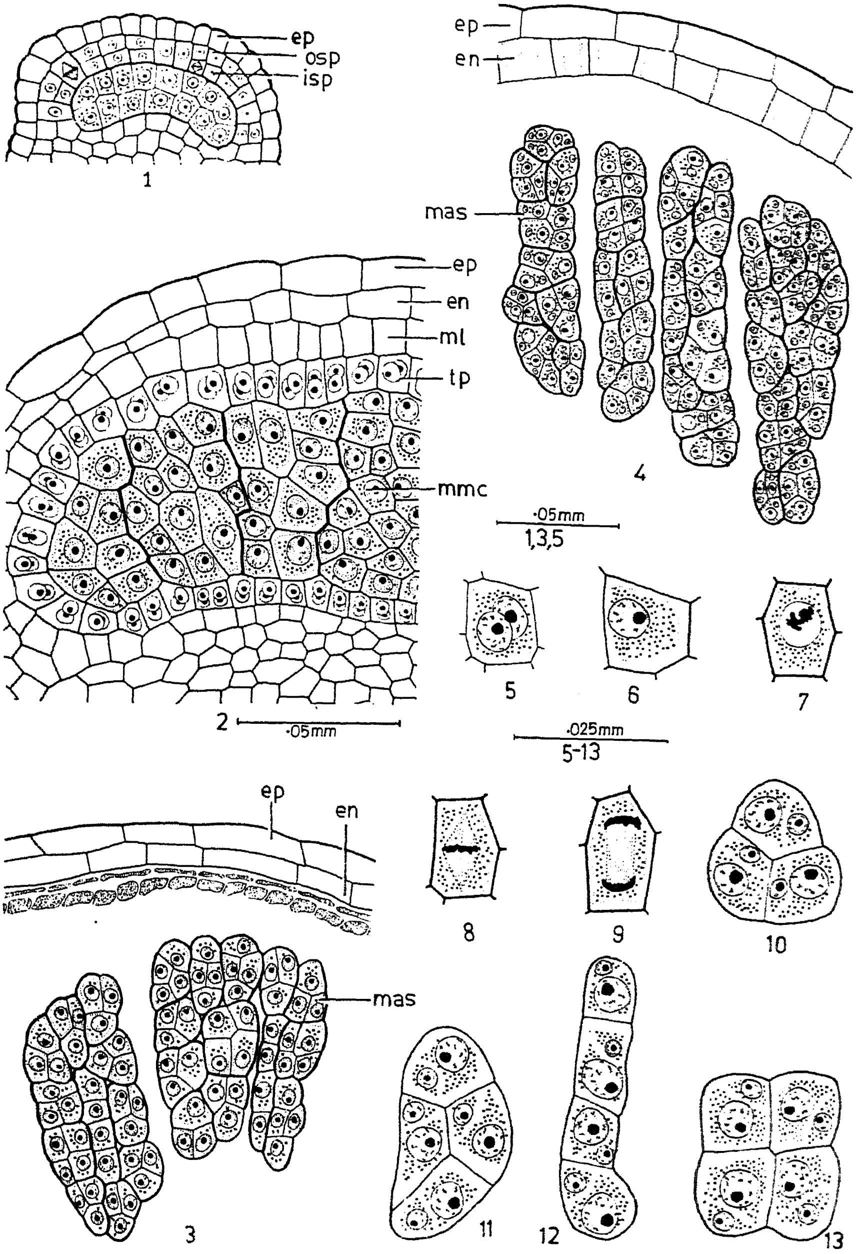
3.3 *Megasporogenesis and female gametophyte*

The hypodermal archesporium is 1-celled and functions directly as the megaspore mother cell (figure 18). It undergoes the first meiotic division to form a dyad with a small micropylar and a large chalazal cell (figure 19). The micropylar dyad cell degenerates (figure 20) whereas the chalazal one undergoes the second meiotic division to give rise to two megaspores of which the lowermost is functional (figure 21). It undergoes 3 mitotic divisions resulting in an 8-nucleate embryo sac (figures 22, 23). The mature embryo sac is typical consisting of an egg apparatus, a secondary nucleus and 3 antipodal cells (figure 24). The development of the female gametophyte conforms to the monosporic, Polygonum type.

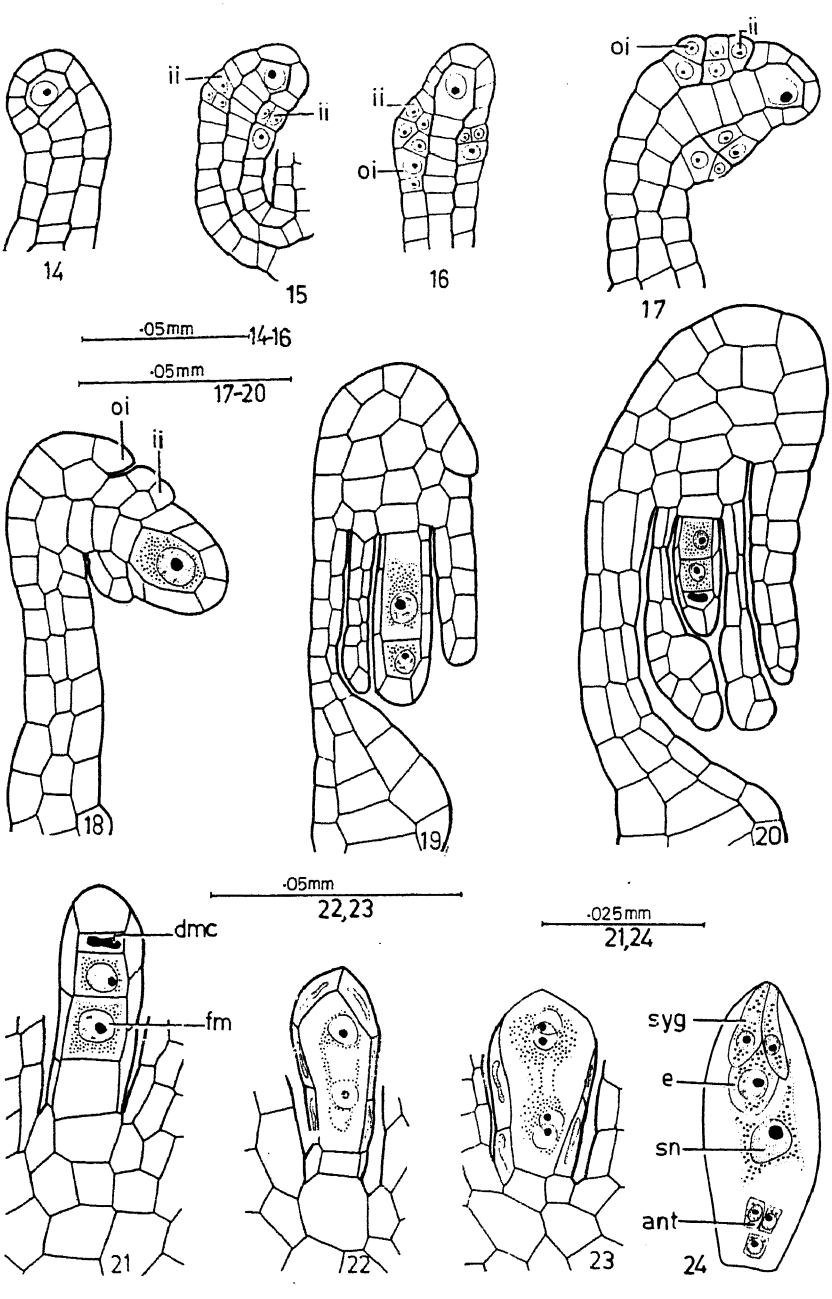
3.4 *Endosperm and embryo*

After fertilization the primary endosperm nucleus initially enlarges (figure 25) but degenerates soon after the proembryonal tetrad stage. The zygote (figure 25) divides transversely to form a terminal cell *ca* and a basal cell *cb* (figure 26). The former divides longitudinally and the latter transversely to form an inverted T-shaped, 4-celled proembryo (figures 27–29). The two cells of *ca* undergo another longitudinal division at right angles to the first to give rise to a quadrant *q* (figure 30) which then divides transversely into an octant with tiers *l* and *l′* (figure 31).

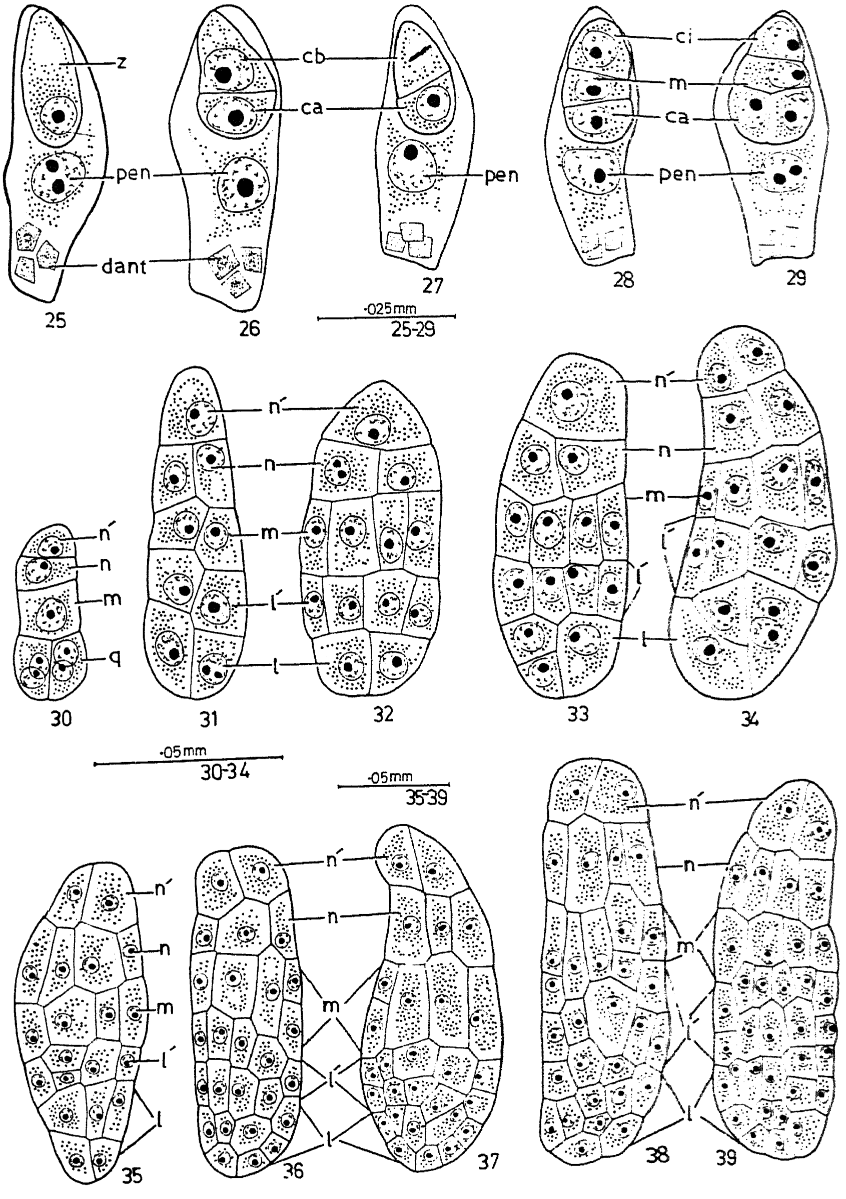
The cell *ci* divides transversely forming two cells *n* and *n′* (figure 30) followed by a longitudinal division in the cells *m*, *n* and *n′* (figures 31, 34). Subsequent divisions



Figures 1–13. Microsporangium, microsporogenesis and male gametophyte in *Goodyera repens*. **1–3.** Portions of transections of developing anthers at differentiation of the wall layers; at microspore mother cell stage and at pollen tetrad stage respectively. **4.** C.S. anther showing epidermis, fibrous endothecium and massulae. **5.** Binucleate tapetal cell. **6–9.** Meiosis in microspore mother cells. **10–13.** Tetrahedral, decussate, linear and isobilateral microspore tetrads. (*en*, Endothecium; *ep*, epidermis; *isp*, inner secondary parietal layer; *mas*, massulae; *ml*, middle layer; *mmc*, microspore mother cell; *osp*, outer secondary parietal layer; *tp*, tapetum).



Figures 14–24. Megasporangium, megasporogenesis and female gametophyte in *Goodyera repens*. **14.** Ovular primordium with hypodermal archesporial cell. **15–17.** Young ovules showing the dermal initiation of inner and outer integuments. **18.** Megaspore mother cell; note the origin of integuments. **19.** Dyad cell showing unequal division. **20, 21.** Triad cells note the degenerated micropylar dyad cell and two megaspores of which the chalazal one is functional. **22, 23.** Two- and 4-nucleate embryo sac. **24.** Organized embryo sac. (*ant*, Antipodals; *dms*, degenerated micropylar dyad cell; *e*, egg; *fm*, functional megaspore; *ii*, inner integument; *oi*, outer integument; *sn*, secondary nucleus; *syg*, synergid).



Figures 25–39. Endosperm and embryogeny in *Goodyera repens*. **25.** Zygote, primary endosperm nucleus and degenerated antipodals. **26.** Two-celled proembryo. **27.** Same; with basal cell in division. **28.** Three-celled proembryo. **29.** Inverted T-shaped proembryonal tetrad. **30, 31.** Seven- and 13-celled proembryos. **32–39.** Stages leading to the formation of an undifferentiated mature embryo. (*dant*, Degenerated antipodals; *pen*, primary endosperm nucleus; *z*, zygote).

occur in both planes in the cells of tiers *l*, *l′*, *m*, *n* and *n′* (figures 32, 33, 35–38), resulting in an undifferentiated embryo (figure 39). It lacks the suspensor.

3.5 *Seed and seed coat*

The seeds are minute, extremely light and numerous. Each seed consists of an undifferentiated embryo which is surrounded by a transparent seed coat (figure 45). During post-fertilization stages the tegmen and the inner layer of testa degenerate. The cells of outer layer of the testa become transparent and form the seed coat.

3.6 *Pericarp*

The ovary (figure 46) in transection shows 3 fertile and 3 sterile valves (figure 40). Each valve consists of one vascular bundle. The fertile valves are broader than the sterile ones. The outer epidermis of both the valves gives rise to uniseriate trichomes (figures 40, 47).

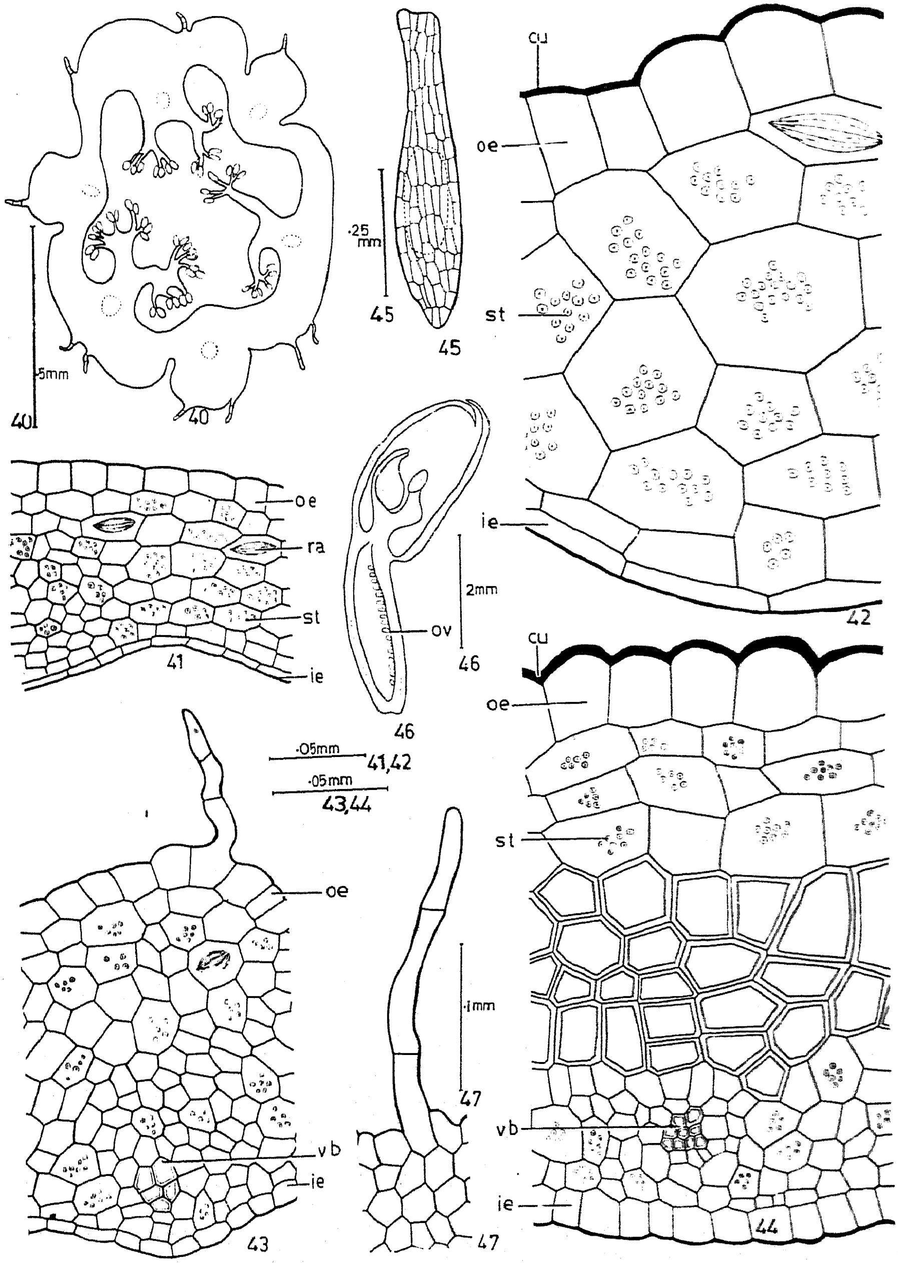
The fertile valve of the young ovary is 7 to 9 cells thick and parenchymatous (figure 41). Some of these cells contain starch and raphides. In the post-fertilization stages, the outer wall of outer epidermis is strongly cutinised. The ground tissue remains parenchymatous (figure 42).

In the pre-fertilization stages the sterile valve consists of 16 or 17 layers of parenchymatous cells (figure 43). Some of the cells contain starch grains and raphides as in the fertile ones. After fertilization, the cells of the sterile valve undergo marked changes. Its ground tissue become demarcated into the outer, middle and inner zones (figure 44). The outer zone consists of 4 layers of thin-walled parenchyma cells. The middle zone is made up of 5 layers of longitudinally elongated sclerenchyma cells. The inner zone is 7 cells thick consisting of small thin-walled cells. The vascular supply passes through the middle of the inner zone. Some cells of the inner and outer zones contain starch grains (figure 44).

4. Discussion

The developmental details of anther wall in *G. repens* are similar to *Neottia listeroides* (Sood 1984) in showing the Monocotyledonous type of development (Davis 1966). However, the other previously worked out taxa of tribe Neottieae, *Zeuxine sulcata* (Seshagiriah 1941; Swamy 1946), *Spiranthes australis* (Maheshwari and Narayanaswami 1952) and *Cephalanthera ensifolia* (Mohana Rao and Sood 1986) differ in possessing 5-layered thick anther wall. Swamy (1949a) based upon his work on *Paphiopedilum druryi* stated that binucleate tapetum is a characteristic feature of Diandrae. However, the occurrence of binucleate tapetal cells in *G. repens*, a monandrous orchid, indicates that it is not an exclusive feature of Diandrae. Although Swamy (1949a) reported that the epidermal layer in the mature anthers of orchids degenerates and the endothecium forms the conspicuous layer, it persists in the present case. At anthesis, the pollen tetrads separate out into individual grains in *C. ensifolia* (Mohana Rao and Sood 1986) and remain together as tetrads in *N. listeroides* and *S. australis* (Maheshwari and Narayanaswami 1952; Sood 1984). On the other hand, in *G. repens*, the pollen tetrads are held together to form small bundles, the massulae, as reported in members of the tribe Orchideae (Mohana Rao and Sood 1979; Sood 1986; Sood and Mohana Rao 1986b).

The initiation and ontogeny of the integuments in *G. repens* is practically similar to that of other investigated taxa of Orchidaceae (see Mohana Rao and Sood 1986;



Figures 40–47. Pericarp and seed coat in *Goodyera repens*. **40.** Transection of ovary showing fertile and sterile valves. **41.** Portion of fertile valve at MMC stage. **42.** Pericarp portion in the region of fertile valve. **43.** Ovary wall in the region of sterile valve. **44.** Pericarp of mature fruit in the region of sterile valve. **45.** Mature seed. **46.** Median l.s. flower. **47.** Uniseriate, epidermal hair. (*cu*, Cuticle; *ie*, inner epidermis; *oe*, outer epidermis; *ov*, ovary; *ra*, raphides; *st*, starch; *vb*, vascular bundle).

Sood 1984, 1986; Sood and Mohana Rao 1986a, b). Afzélius (1916) reported the formation of a linear tetrad of megaspores in *G. repens* although observations on the same taxon, clearly shows the formation of a triad row of cells as reported earlier in most of the taxa of Neottieae (Abe 1971; Mohana Rao and Sood 1986).

Conflicting opinions exist regarding the presence or absence of suspensor and the type of embryogeny in the genus *Goodyera.* Treub (1879) reported the occurrence of linear proembryonal tetrad and excessively elongated 1-celled suspensor haustorium in *G. discolor.* Leavitt (1901) stated that the embryos of *G. pubescens* and *G. tesselata* seem to lack the suspensor. The present study on *G. repens* clearly shows the formation of an inverted, T-shaped proembryonal tetrad; participation of all the derivatives of basal and terminal cells in the organisation of mature embryo; absence of suspensor haustorium and the Asterad type of embryogeny of Swamy (1949b). As in most of the orchids studied, with a few exceptions (see Veyret 1965) the mature embryo of *G. repens* lacks differentiation into histogens.

Acknowledgements

The author wishes to express his gratefulness to Prof. H O Agrawal for his kind interest in this study and to Prof. P R Mohana Rao, Nagarjuna University, Guntur, for his valuable suggestions.

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Rhythmic oscillations in wheat chloroplast photochemical activity. I. Oscillations in whole chain, photosystem II and photosystem I electron transport activities

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Abstract. Chloroplasts isolated from the primary leaves of wheat seedlings grown under different light in the laboratory as well as from the leaves of field grown seedlings exhibited distinct rhythmic oscillations in photosynthetic electron transport activities. Chlorophyll content in the primary leaves of continuous light grown wheat seedlings did not oscillate. Whole chain electron transport and partial reactions measured as photosystem II and photosystem I activities estimated at regular intervals oscillated with a periodicity of about 12–16 h. The periodicity of less than 22 h suggested that the rhythms are of a non-circadian type. The oscillations in photochemical activity persisted whether the seedlings were raised under continuous light or under light-dark cycles, indicating their endogenous nature. The photochemical activities attained maximal levels during both the light and dark phases of the cycles. The rhythm pattern persisted in the presence of photophosphorylation uncouplers. A time dependent sensitivity of uncouplers to stimulate electron transport activity was observed. The timings of the activity maxima for photosystem II and photosystem I mediated reactions alternated each other during the day. The ratios of photosystem II to photosystem I activities were out of phase of photosystem I and closely resembled the rhythm pattern in photosystem II activity indicating a possible oscillation in the population of active photosystem units. The results suggest that the temporal modulation of the photochemical activity in wheat chloroplasts is brought about by some sort of reversible conformational change in the thylakoid membranes.

Keywords. Chloroplast activity; electron transport; endogenous rhythms; gramicidin; photosystems I and II; *Triticum aestivum*.

1. Introduction

Rhythmic phenomena in the photosynthetic light reactions of a number of algae and some higher plants have been reported (Lonergan 1984b). O_2 evolution measured in intact cells of algae such as *Euglena*, *Gonyaulax* and *Acetabularia* (Edmunds 1984; Lonergan and Sargent 1978) and in intact mesophyll cells of *Pisum* (Lonergan 1981) oscillated rhythmically over 24 h. A rhythm in the whole chain electron transport measured as water to methylviologen assay has also been reported for many systems (Lonergan and Sargent 1979; Lonergan 1981, 1984a; Samuelsson *et al* 1983). While many algal species did not show any oscillation in the chlorophyll (Chl) content (Prezelin and Matlick 1980; Harding *et al* 1981), a few others have been shown to exhibit diurnal changes in their cellular pigment contents (Laval-Martin *et al* 1979; Lonergan and Sargent 1979; Owens *et al* 1980).

Many attempts have been made to identify if any of the partial photochemical reactions, such as photosystem II (PS II) or photosystem I (PS I) activity also oscillated rhythmically. Most of the studies on *Euglena*, *Acetabularia* and legume chloroplasts showed no oscillations in the activity of PS II and PS I catalyzed reactions (Lonergan and Sargent 1979; Lonergan 1981; Vanden Driessche and Lannoye 1982). However, using the cell free extracts of *Bryopsis maxima* (Okada and Horie 1979) and *Gonyaulax polyedra* (Samuelsson *et al* 1983), PS II Hill activity has been demonstrated to be rhythmic. Uncouplers of photophosphorylation have been used in the study of electron transport rhythms, in order to avoid the complications of differentially coupled chloroplast preparations. The uncouplers were found to have a differential and time-dependent effect on the chloroplast membranes (Lonergan 1981). The photosynthetic light reactions are generally accepted to be the site of rhythm control. Studies on the photochemical reactions and Chl*a* fluorescence yield (Prezelin and Sweeney 1977; Okada and Horie 1979; Lonergan 1981) have indicated that the rhythmicity is generated due to some changes in the thylakoid membrane properties. However, the site(s) of origin of the rhythmicity in the electron transport chain have not been fully elucidated.

In the present communication an attempt has been made to study the presence of oscillations in various photochemical parameters of wheat chloroplasts isolated from field and laboratory grown seedlings. Distinct oscillations in PS II catalyzed O_2 evolution, PS I, whole chain electron transport activities and differential expression of oscillations in photochemical activities in the presence and absence of a photophosphorylation uncoupler gramicidin have been observed. The results suggest that rhythmic changes in the physical state of the thylakoid membranes induce oscillations in the electron transport activity.

2. Materials and methods

2.1 *Growth conditions*

Seeds of *Triticum aestivum* L. cv. Kalyansona obtained from the Indian Agricultural Research Institute, New Delhi, were germinated on 3 layers of moist germination paper in petri dishes. Illumination was provided by a bank of 40 W Phillips cool daylight fluorescent lamps giving $\simeq 20\ \mathrm{W\,m^{-2}}$ irradiance at the level of the petri dishes at $25 \pm 1°\mathrm{C}$. Conditions for the continuous light (LL) grown plants included constant irradiance throughout the 15 day long growth period under constant temperature ($\sim 25°\mathrm{C}$). Where the growth was under artificial light-dark (LD) cycles, a dark period of 12 h was provided. Laboratory grown plants were supplied with half strength Hoagland nutrients twice in 12–15 days. For experiments on field grown plants, seedlings were grown in the nursery in winter season (January–March) under natural conditions. The daily photoperiod was approximately equal to 12 h and the temperature fluctuations (minimum to maximum) in this season ranged from 6–25°C. Leaves harvested at regular 4 h intervals were immediately brought to the cold room for chloroplast isolation.

2.2 *Chloroplast isolation*

Fully expanded primary leaves (12–15 days old) and fifth and sixth leaves from about 60 days old field grown plants were used in all the experiments. Chloroplasts were isolated using about 30 primary leaves or about 12 leaves from field grown plants as described previously (Sayeed *et al* 1983). The isolation buffer contained 5 mM N-tris (hydroxymethyl) methyl aminoethane-sulfonic acid (TES) (pH 7·8), 0·4 M sucrose and 1 mM Na_2 EDTA. All operations were carried out at 4°C under dim green light. Estimation of Chl from isolated chloroplasts as well as from leaf segments was carried out following the method of Arnon (1949) using 80% cold acetone for extraction of Chl. For pigment estimation on unit weight basis multiple samples were drawn randomly from a population of seedlings and estimated individually. Estimation of pigments and measurements of photochemical activities were started at a time that coincided with the beginning of a photoperiod in all cases. Sunrise time in the standard clock was marked as zero hour in the figures.

2.3 *PS II assay*

Partial electron transport activity of isolated chloroplasts mediated by PS II resulting in oxygen evolution was measured polarographically using a Clark-type oxygen electrode (Sayeed *et al* 1985). Chloroplast samples were illuminated with the help of a 300 W projector lamp providing saturating incandescent light ($\simeq 450\ \mathrm{W\ m^{-2}}$). Three ml of the reaction mixture contained 50 mM TES buffer (pH 7·5), 5 mM $MgCl_2$, 10 mM KCl, 1 mM $K_3Fe(CN)_6$, 100 μm phenylene diamine (PDox) and when present the concentration of gramicidin-S was 5 μM (final concentration of ethanol in the reaction medium did not exceed 1%). Chloroplast samples equivalent to 40–60 μg Chl were taken for each estimation.

2.4 *PSI and whole chain electron transport assays*

PS I mediated electron transport activity was estimated as a measure of oxygen uptake due to methyl viologen (MV) reduction by 2, 6-dichlorophenol indophenol (DCPIP) and sodium ascorbate donor couple. The experimental setup was the same as described for PS II assay. Three ml of the reaction mixture contained 50 mM TES buffer (pH 7·5), 5 mM $MgCl_2$, 10 mM KCl, 100 μM DCPIP, 2 mM sodium ascorbate (Asc), 0·5 mM MV, 1 mM sodium azide and 10 μM DCMU. Whole chain electron transport (H_2O to MV) activity was measured as in PS I assay. The reaction mixture (pH 7·5) contained everything as in the assay mixture for PS I measurement except DCPIP, ascorbate and DCMU. Gramicidin-S was used at a final concentration of 5 μM. Chloroplast samples equivalent to 40–60 μg Chl were used for each estimation.

Irradiance was measured using a YSI model 65 A radiometer (YSI, Ohio, USA). All the chemicals used were of high purity obtained from commercial sources and used without any further purification.

3. Results

3.1 *Lack of oscillation in Chl contents*

Chl content of the primary leaves from continuous light grown wheat seedlings estimated at different times of day and expressed per gram fresh weight was shown in figure 1. The total Chl did not exhibit any specific pattern of oscillation over the two day long period of investigation, but showed minor and inconsistent fluctuations

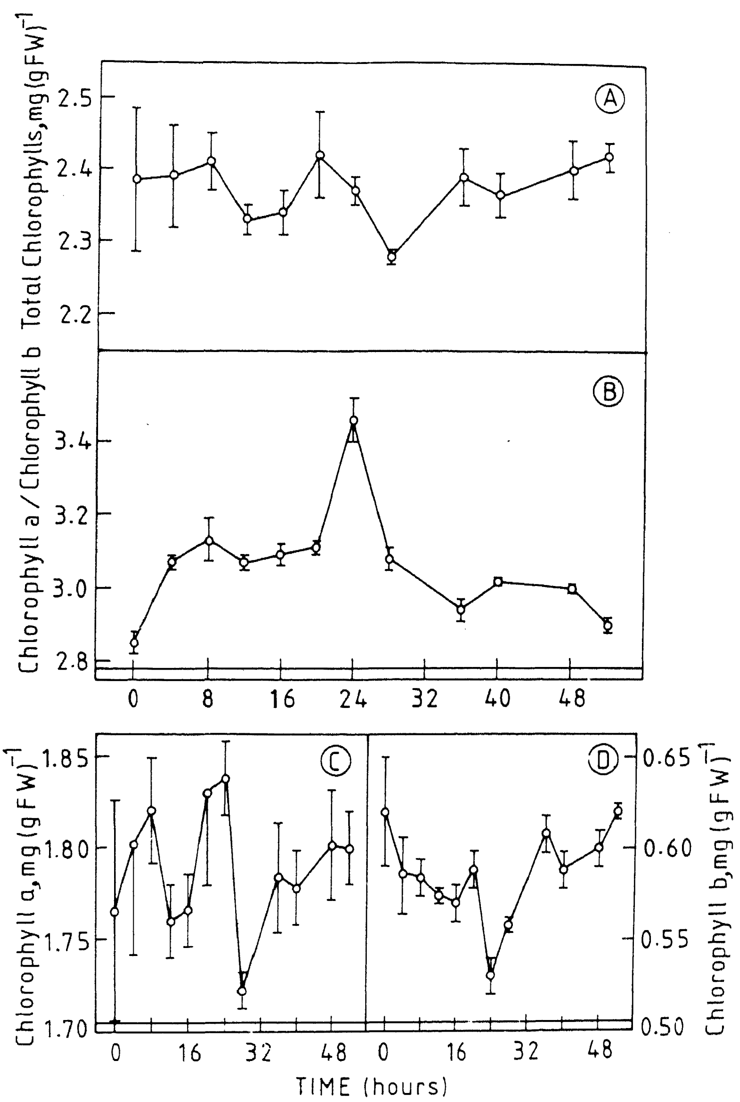

Figure 1. Chlorophyll content of the primary leaves sampled at different times of the day from continuous light grown 12–15 day old wheat seedlings. Sunrise time in the standard clock was marked as 0 h of estimations. **A.** Total chlorophyll content. **B.** Chl *a* Chl *b* ratios. **C.** Chl *a* content. **D.** Chl *b* content. The mean represents 9 replicates.

with a minimum to maximum difference of less than 6% (figure 1A). Fluctuations in Chl *a* and Chl *b* (figure 1C–D) as well as variations in the ratios of Chl *a* to Chl *b* (figure 1B) were also random. We, therefore, assume that the Chl content of the leaves remained more or less constant throughout the day.

3.2 *Oscillations in whole chain electron transport*

Chloroplasts isolated from the primary leaves of wheat seedlings grown in the laboratory under continuous light (LL) or light-dark cycles (LD) or under LL condition after an initial LD entrainment exhibited rhythmic oscillations in their whole chain electron transport activity measured as H_2O to MV assay (figure 2). LL grown seedlings showed an oscillation pattern with a periodicity of about 12 h in the basal electron transport (in the absence of uncouplers) and the amplitude of the rhythm ranged from 1·3–2·1 fold (figure 2A). Similar pattern of 12 h rhythm in the whole chain electron transport was also observed in the chloroplasts of LD grown seedlings (figure 2B). The peak timings in both the cases were more or less comparable, with peaks appearing in the day as well as night phases of the cycles in LD grown plants, and the subjective light and dark periods of the LL grown plants. The seedlings entrained by LD cycles till the beginning of the experiment upon transferring to continuous light condition as that of LL growth condition also retained the oscillation in their electron transport performance involving the entire

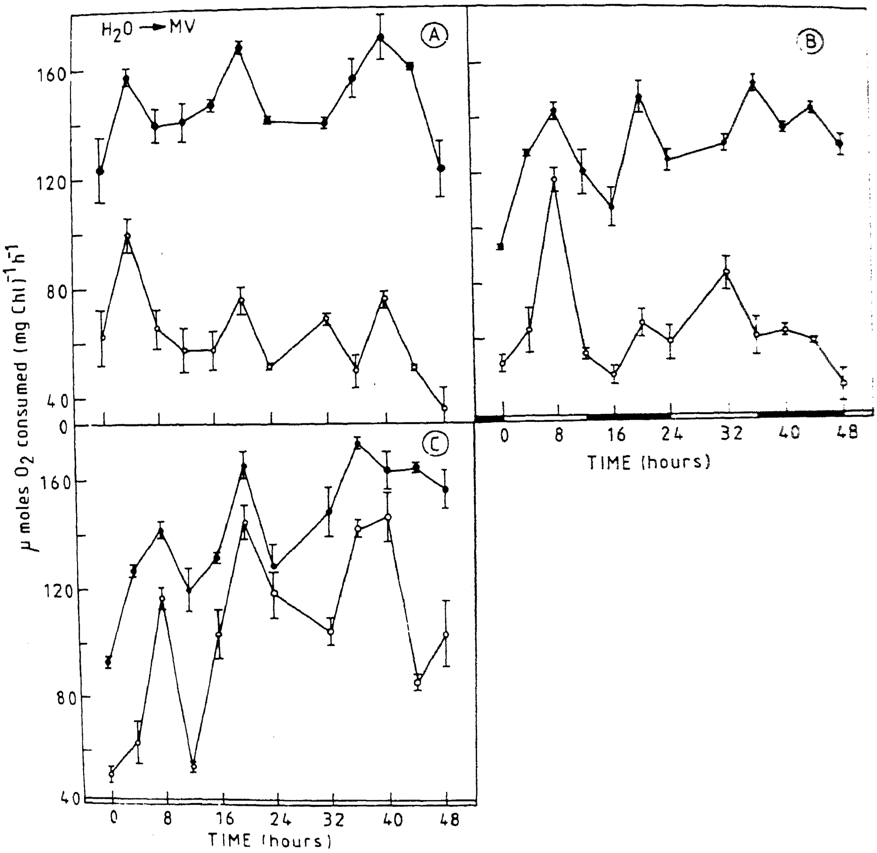

Figure 2. Oscillations in whole chain electron transport activity of the chloroplasts isolated from the primary leaves of 12–15 day old wheat seedlings. Whole chain electron flow was measured as H_2O to MV assay at 4 h intervals. Sunrise time in the standard clock was taken as 0 h of the measurements. **A.** LL grown plants. **B.** LD grown plants. **C.** LD entrained plants grown in LL. (○), Basal rates; (●), uncoupled rates (with gramicidin). Night time is represented by the solid horizontal bars. The mean represents 8 replicates.

chain (figure 2C). The peak timings were comparable with those in LD grown plants and the amplitude of the rhythm varied between 1·4 and 2·7 fold. There was, however, an uncertainty of about 4 h in the peak timings of the oscillations among the various growth conditions (figure 2A–C).

Exogenously added chemicals such as uncouplers of photophosphorylation, herbicides and phytohormones have been shown to influence the expression of the photosynthesis rhythms (Lonergan 1981; Vanden Driessche 1984). Addition of 5 μM gramicidin-S to the reaction medium did not alter the oscillatory behaviour of the whole chain electron transport in any of the growth conditions (figure 2A–C). The electron transport activity in the presence of gramicidin-S exhibited an enhancement at all the measurement points. The peak timings of the uncoupled electron transport rhythms were comparable to those measured in the absence of the uncoupler, though the occasional 4 h difference was observed. The degree of uncoupler mediated enhancement in the activity was higher in the LL and LD grown plants (figure 2A, B) as compared to the LD entrained plants maintained in LL (figure 2C). The gramicidin effect was not uniform at all times of the day and greater extent of enhancement in the activity was seen at times when the basal activity was at the minimal level (figure 2).

3.3 *Rhythm in PS II mediated oxygen evolution*

O_2 evolution mediated by PS II was estimated using the H_2O to PD_{ox} + FeCN assay system, which due to effective mediation of electron flow by phenylenediamine to ferricyanide, constitutes a reliable PS II measurement (Saha *et al* 1971). The PS II catalyzed O_2 evolution measured at regular intervals using chloroplasts isolated from leaves of either field or laboratory grown plants oscillated rhythmically similar to the whole chain electron transport. Oscillations of about 8–12 h period were observed in the PS II mediated O_2 evolution activity in chloroplasts isolated from the field grown leaves (figure 3), with the activity maxima appearing in the day as well as in the night phases of the cycle. PS II catalyzed O_2 evolution monitored in the presence of gramicidin-S yielded well defined oscillations which were in phase with those of the basal rates measured without the uncoupler. Gramicidin mediated enhancement in O_2 evolution was observed throughout the day. However, differential sensitivity to the uncoupler during the observation period was observed. Only at certain times of the day did the gramicidin mediated enhancement become significant (see figure 3 inset).

Oscillations with periods of about 12 h were observed in the PS II catalyzed O_2 evolution activity in chloroplasts isolated from the seedlings grown in the laboratory under continuous light ($\simeq$20 W m^{-2}) and constant temperature (25° C; figure 4). The basal activity exhibited activity maxima during the presumptive day and night phases with the peak timings comparable to those of the field grown plants. The presence of gramicidin in the reaction medium brought about a different type of response in the chloroplasts isolated from LL grown plants (figure 4). Almost throughout the day gramicidin exerted an inhibitory effect on O_2 evolution in this case (figure 4, inset) in contrast to the observation in the field grown plants. Other uncouplers such as ammonium chloride and carbonyl cyanide 4-(trifluoroxy) phenylhydrazone (FCCP) also elicited a similar response (data not shown).

When the seedlings were raised under an artificial cycle of 12 h in the laboratory

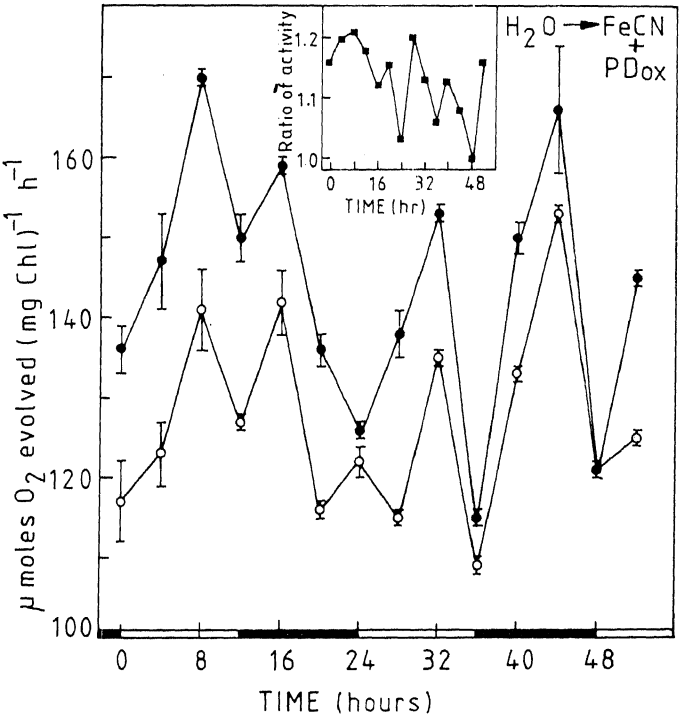

Figure 3. Oscillations in PS II catalyzed O_2 evolution (H_2O to PD_{ox} + FeCN) in chloroplasts isolated from the leaves of field grown wheat seedlings at various times of day. (○), Basal rates; (●), uncoupled rates with 5 μM gramicidin. Sunrise time in the standard clock was taken as 0 h and the night time is represented by the horizontal bars. Inset shows the ratio of mean of activity with and without gramicidin-S. The mean represents 6 replicates.

growth conditions, the PS II mediated O_2 evolution activity exhibited a periodicity slightly different from that of the field grown or LL grown seedlings (figure 5). The period of oscillations was about 16 h and the activity peaks appeared in the early or late hours of the light phase in the LD cycle. Oscillations in the activity measured in the presence of gramicidin were in phase with those in the basal activity and exhibited the uncoupler mediated enhancement in the O_2 evolution throughout the day (figure 5), similar to the observation in the field grown plants. However, the extent of gramicidin induced enhancement was smaller than that in the field grown plants (figure 5, inset).

Transfer of LD grown plants at the beginning of the experiment to continuous light condition as described for LL plants resulted in an altered pattern of rhythmicity in the PS II mediated O_2 evolution (figure 6). The period of the oscillation was about 16–20 h and the peaks were observed in the subjective night phase and in the late day phase of the cycle, contrary to the peaks observed in the light phases of the cycles in the case of LD grown plants. As seen in the LD grown seedlings, the gramicidin mediated enhancement was small at most times and even a slight inhibition was observed at certain points of the measurement (figure 6, inset). The amplitude of the rhythms in PS II mediated O_2 evolution in all the growth conditions ranged between 1·2 and 1·5 fold.

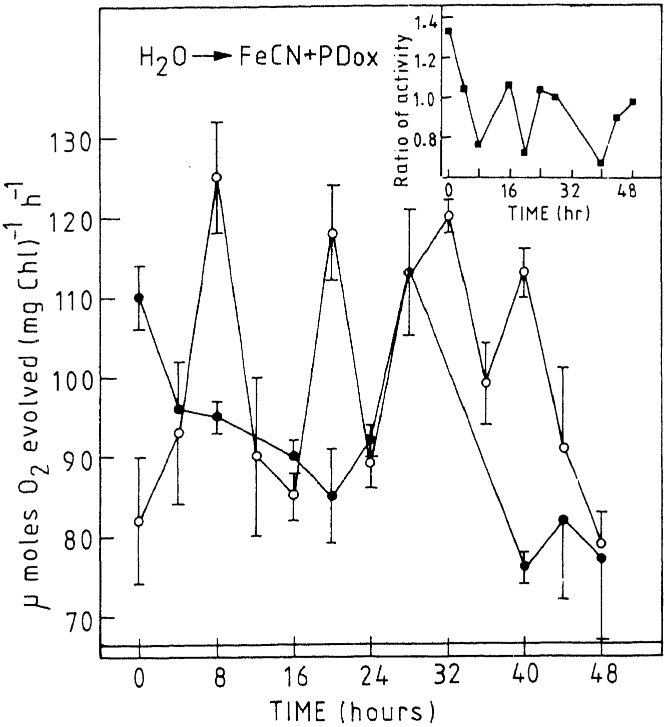

Figure 4. Oscillations in PS II catalyzed O_2 evolution (H_2O to PD_{ox} + FeCN) in chloroplasts isolated from the primary leaves of continuous light grown seedlings. (○), Basal rates (without gramicidin); (●), uncoupled rates with gramicidin-S. Sunrise time was taken as 0 h of the measurements. Inset shows the ratio of the mean activity with and without gramicidin-S. The mean represents 10 replicates.

3.4 *Rhythm in PS I mediated electron transport*

Oxygen uptake due to photoreduction of MV and its subsequent autooxidation, measured as a PS I assay was also rhythmic in wheat chloroplasts. Oscillations in PS I activity of chloroplasts isolated from field grown plants measured without gramicidin (figure 7) showed peaks with periods of about 8–20 h. While the major peaks appeared in the dark phase, only minor peaks of activity were noted in the beginning of the light phase of each cycle. Rhythmic oscillations of a similar type were also observed in the LL grown plants (figure 8) but the oscillations in the basal activity (without gramicidin) showed a periodicity of about 16 h and reached a maximum either in the presumptive night or day period of the cycle. In the case of LD grown plants the PS I mediated electron flow showed frequent oscillations with periods of about 12 h and the activity maxima appeared both in the light and dark phases (figure 9) of the LD cycle. This pattern of frequent oscillations in PS I activity was retained by LD entrained plants grown in LL (figure 10) with period lengths ranging from 8–12 h. In all the growth conditions the amplitude of the rhythmic change in PS I activity varied between 1·4 and 2·6 fold.

Gramicidin mediated enhancement in PS I activity was observed throughout the period of investigation in all the growth conditions (figures 7–10). When gramicidin

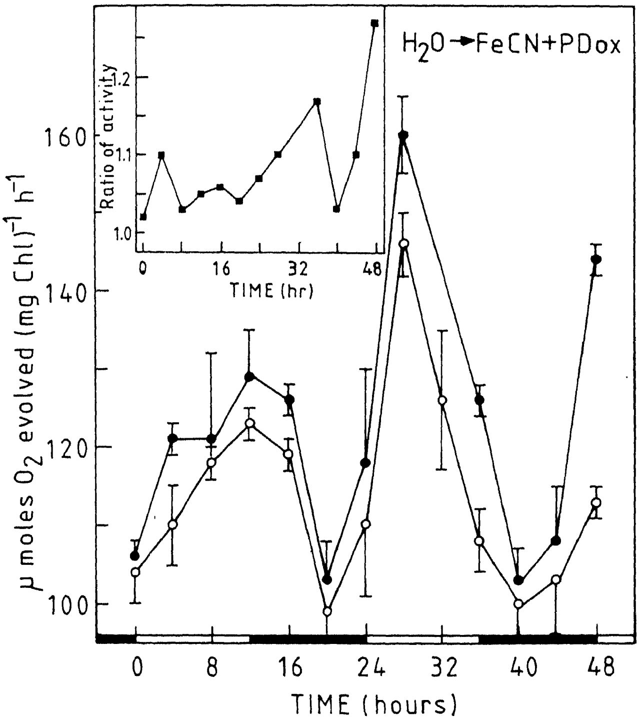

Figure 5. Oscillations in PS II catalyzed O_2 evolution in chloroplasts isolated from the primary leaves of light-dark grown seedlings. (○), Basal rates (without gramicidin); (●), uncoupled rates with gramicidin. Sunrise time was taken as 0 h of the measurements. Inset shows the ratio of the mean activity with and without gramicidin. The mean represents 8 replicates.

was added to the reaction medium, PS I activity in the chloroplasts isolated from field grown plants as well as from LL grown plants exhibited additional peaks (figures 7, 8). These new peaks appeared in the light phases of the cycles or in the presumptive light period of LL grown plants. The time dependent changes in the response of the thylakoids to gramicidin are shown in the insets of figures 7 and 8. Greater degree of enhancement in the activity was noted mostly when the basal activity was at lower levels. Similar effect on PS I activity in LL grown plants was also observed in the case of ammonium chloride and FCCP treatments (data not shown). Appearance of additional peaks in PS I activity in the light phase of the cycle or disappearance of existing peaks from dark phase upon addition of gramicidin has been observed in LD grown as well as in LD entrained LL grown plants (figures 9, 10). The highest sensitivity of the thylakoid membranes to gramicidin treatment was seen mostly during the light phase of the cycle as shown by the ratios of PS I activity measured with and without the uncoupler (figures 9 and 10, insets). However, the PS I activity rhythms in the presence of gramicidin were not always in phase with the basal activity oscillations, contrary to the trend observed in the case of PS II mediated O_2 evolution rhythms owing to the appearance or disappearance of activity maxima.

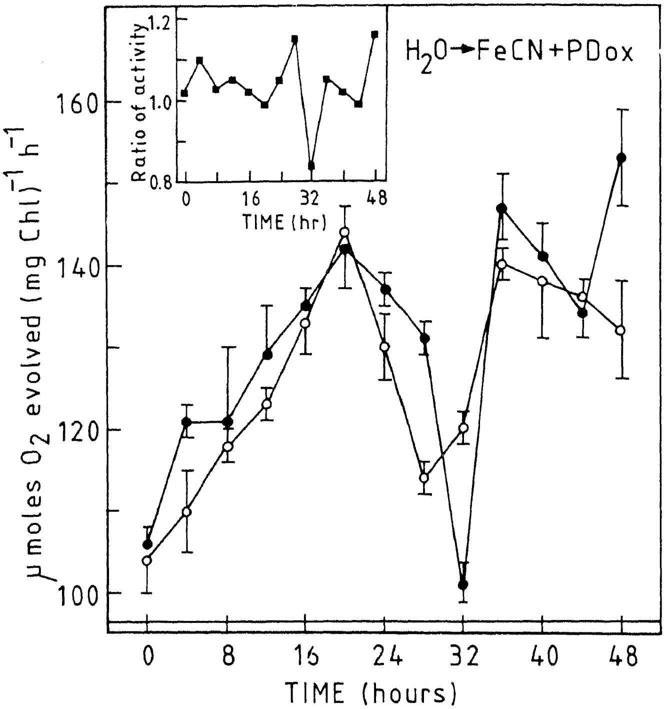

Figure 6. Oscillations in PS II catalyzed O_2 evolution in chloroplasts isolated from the primary leaves of seedlings grown under LD cycles and subsequently transferred to continuous light at the beginning of the experiment. (○), Basal rates; (●), uncoupled rates with gramicidin. Inset shows the ratio of the mean activity with and without gramicidin-S. The mean represents 8 replicates.

3.5 *Oscillation in PS II to PS I activity ratio*

Since the oscillations in PS I catalyzed activity did not always appear to be in phase with those in PS II activity, we plotted the ratios of PS II to PS I activity. Figure 11 shows the rhythmic changes in the ratios of PS II mediated O_2 evolution to PS I mediated O_2 uptake activity of chloroplasts isolated from the seedlings raised in different growth conditions. Oscillations in the ratios of PS II/PS I activities in chloroplasts isolated from field grown plants showed a periodicity of about 12–16 h and the timing of the peaks coincided with that of the PS II catalyzed O_2 evolution peaks observed in the light phase of the cycles (figure 11A). With gramicidin, these activity ratios showed oscillations in phase with those seen in the ratios of basal activity but the amplitude was highly reduced. LL grown plants exhibited 3 peaks and a minor shoulder in the PS II/PS I activity ratios (figure 11B) with a 12 h period and the peak timing closely correlated with that of the PS II mediated O_2 evolution oscillations (see figure 4). The ratios of photosystem activities measured in the presence of gramicidin, however, did not oscillate in phase with the ratios of basal activity, but the amplitude was not reduced significantly. The oscillations in PS II/PS I activity ratios in the case of LD plants exhibited two major and two minor peaks with a periodicity of about 12 h and their major peak timings (figure 11C) were

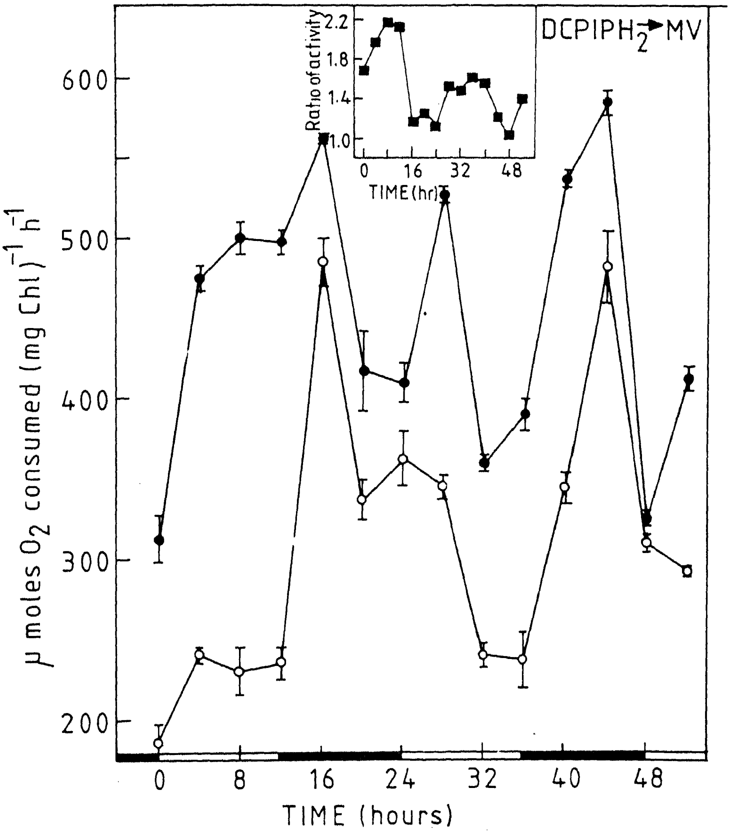

Figure 7. Oscillations in PS I (DCPIPH$_2$ to MV) activity measured in the absence (○) and presence (●) of gramicidin-S in chloroplasts isolated from the leaves of field grown seedlings. Other conditions as in figure 3. Inset shows the ratio of the mean activity with and without gramicidin.

similar to those seen in the O$_2$ evolution pattern. The ratios of uncoupled activity oscillated rhythmically in phase with the basal activity ratios though the amplitude was highly reduced.

A very different pattern of oscillations became obvious when the ratios of basal activity in LD entrained plants grown in constant light (figure 11D) were plotted. The oscillations not only assumed a periodicity of 8–12 h but also showed no resemblance to the rhythm pattern in O$_2$ evolution as observed in the other cases. On the other hand, the gramicidin mediated PS II/PS I activity ratios resembled oscillations in PS II mediated O$_2$ evolution, except that the amplitude was reduced in all the above described oscillations in the ratios it is important to note that not only the resultant oscillation patterns (figure 11A–D) exhibited a close correlation with that of the PS II rhythms, with an exception in LD entrained plants grown in LL (figure 11D) but also resembled the inverted image of the respective PS I oscillation patterns (figures 7–10). The peak timings for PS II and PS I activities alternated with each other such that an out-of-phase oscillation pattern could be visualized for the partial photochemical reactions.

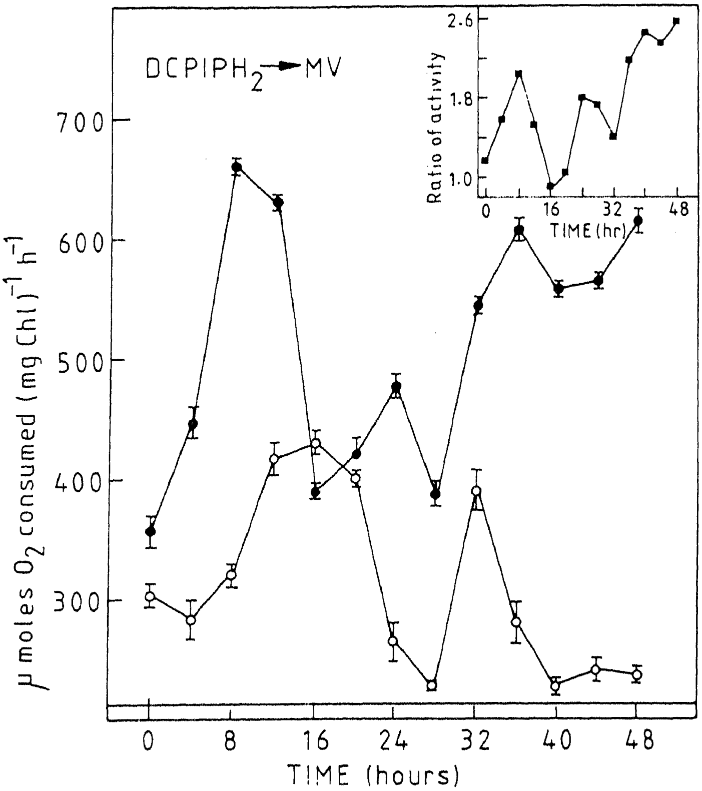

Figure 8. Oscillations in PS I activity measured in the absence (○) and presence (●) of gramicidin-S in chloroplasts isolated from the primary leaves of LL grown seedlings. Other conditions as in figure 4. Inset shows the ratio of the mean activity with and without gramicidin.

4. Discussion

Chloroplasts isolated from the primary leaves of wheat seedlings grown under the specified laboratory conditions as well as from the field grown plants exhibited rhythmic oscillations in their photosynthetic light reactions. Results described here, constitute the first observation of such oscillations in wheat, a C_3 cereal crop plant.

Whole chain electron transport monitored as water to MV assay manifested a distinct pattern of rhythm in chloroplasts isolated from plants grown under LD or constant light conditions. This observation is in agreement with a number of reports existing in the literature on the whole chain electron transport rhythms (Lonergan and Sargent 1979; Lonergan 1981; Samuelsson *et al* 1983). The 12–16 h oscillation in whole chain electron transport was not influenced by the constant bright light (~ 20 W m^{-2}) provided either from the time of seed germination without any dark interval or after a period of entrainment in LD. The persistent rhythm under constant light and temperature conditions suggests that it is endogenous in nature. Contrary to the generally held opinion that rhythmic oscillations tend to disappear in constant bright light conditions (Hastings *et al* 1961; Lonergan and Sargent 1978),

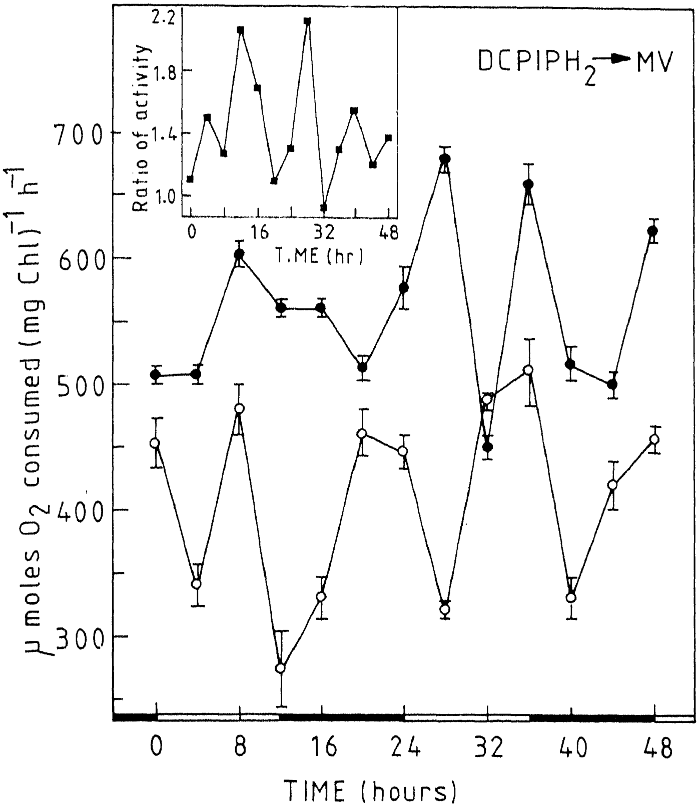

Figure 9. Oscillations in PS I activity measured in the absence (○) and presence (●) of gramicidin in chloroplasts isolated from the primary leaves of LD grown seedlings. Other details as in figure 5. Inset shows the ratio of the mean activity with and without gramicidin.

the oscillations to persist in the wheat chloroplast photochemical activities under continuous bright light conditions were consistently observed. Where dim LL was used to demonstrate the endogenous origin of the photosynthesis rhythms, the irradiance was reduced to about 50% (Prezelin *et al* 1977; Govindjee *et al* 1979) or to less than 4% (Lonergan and Sargent 1979; Lonergan 1981) to that of the growth illumination. In our conditions, however, the growth irradiance was always maintained constant at ~20 W m^{-2}. Similar to our observation, Terbrough and McLeod (1967) have demonstrated a persistent rhythmicity in O_2 evolution in *Acetabularia* cells under constant high light conditions. It is however, not clear why the oscillations are maintained in bright light only in some systems, while in most others rapid damping was observed.

Photochemical activity of the isolated wheat chloroplasts measured as partial electron transport reactions catalyzed by PS II and PS I has also yielded rhythmic oscillations in field and laboratory growth conditions. However, the period length and amplitude of the oscillations observed in the plants grown in diurnal LD cycles in the field or in the laboratory as well as those grown in constant light conditions showed considerable differences. Rhythm in PS II supported O_2 evolution activity showed a periodicity of about 8–12 h in the field grown plants (figure 4). However,

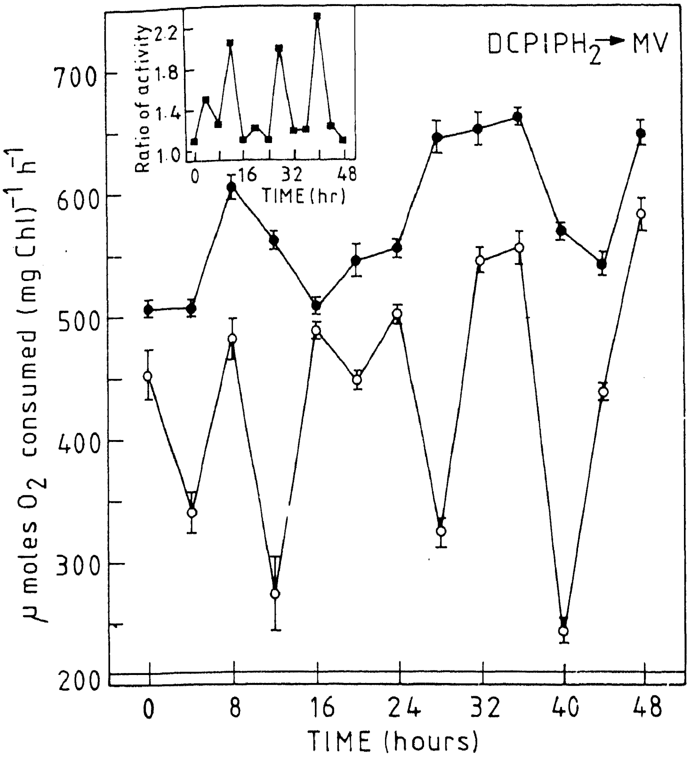

Figure 10. Oscillations in PS I activity measured in the absence (○) and presence (●) of gramicidin in chloroplasts isolated from the primary leaves of LD entrained seedlings subsequently grown in LL. Other details as in figure 6. Inset shows the ratio of the mean activity with and without gramicidin.

the LD and LD entrained LL grown plants showed a period of about 16 h (figures 5, 6). In all the cases the peak activity was observed in the light and dark phases of the cycle or in the subjective light or dark phases of the LL grown plants. However, the minimum to maximum variation in the activity was almost similar in all the conditions, being 1·2–1·5 fold. Though many attempts have been made to ascertain if the rhythmicity observed in the whole chain electron transport was manifested also in the partial reactions such as PS II activity have yielded negative results (Lonergan and Sargent 1979; Lonergan 1981), there are two reports that indicate the possibility of an oscillation in PS II activity (Okada and Horie 1979; Samuelsson *et al* 1983). The observations further indicate that the electron transport pertaining to PS II indeed oscillate rhythmically.

For the first time an oscillation pattern was observed in the electron transport activity mediated by PS I in isolated chloroplasts (figures 7–10). These results suggest that the rhythmicity in whole chain electron transport is generated by the oscillations in partial reactions and that the steps responsible for the whole chain rhythm may be localized in small segment(s) of the electron transport chain. The period length of the PS I oscillations ranged from 8–20 h in various growth conditions with a peak to valley ratio of 1·3–2·4. The frequency of the oscillations increased in LD and LD entrained LL grown plants as compared to the LL plants. Activity maxima appeared

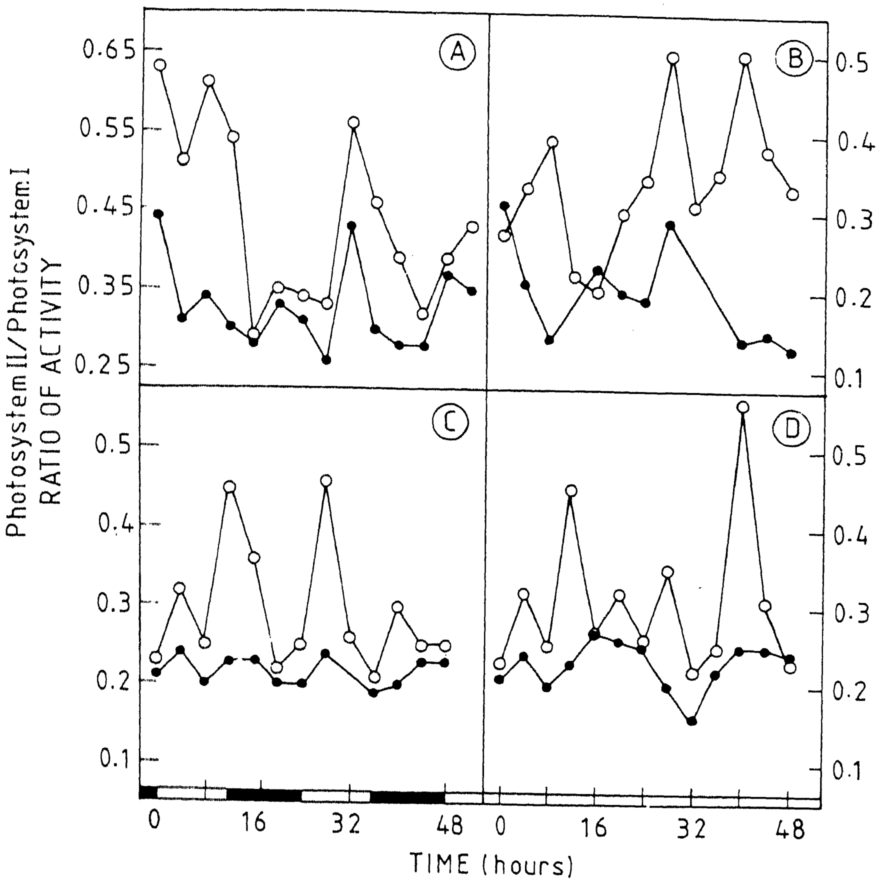

Figure 11. Relative ratios of PS II to PS I activities of the chloroplasts isolated from wheat leaves grown under different conditions as a function of time of day. Assays were performed under saturating light intensity as basal and uncoupled rates. Ratios of basal (without gramicidin) electron transport activity, (○) and uncoupled (with gramicidin) electron transport activity, (●) were calculated from the mean values of 6–10 replicates drawn from 3–4 individual experiments. **A.** Field grown plants. **B.** LL grown seedlings. **C.** LD grown seedlings. **D.** LD entrained seedlings subsequently transferred to LL.

both the light and dark phases of the cycle. However, in the other organisms own to exhibit oscillations in whole chain electron transport (Lonergan and rgent 1979; Lonergan 1981; Samuelsson *et al* 1983) and in PS II activity amuelsson *et al* 1983), the PS I activity did not yield discernible changes over e day.

In our study, the rhythmic changes in whole chain electron transport and PS II or S I partial assays always exhibited a periodicity of less than 24 h. Most of the ythms in photosynthesis hitherto reported have been demonstrated to be circadian nature with a period approximately equal to 24 h (Lonergan 1984b). The cillations observed in wheat, however, seem to be non-circadian since the period ngth is far less than the characteristic limit of circadian rhythms yet they are dogenous in nature. Reports are also available on similar ultradian rhythms eriod < 20 h) in a number of systems (Wagner 1977).

The oscillation pattern seen in the case of PS II mediated O_2 evolution was closely comparable to that observed in whole chain electron transport suggesting the possibility of these rhythmic oscillations being regulated by a common mechanism. However, it is important to note that the oscillations in PS I activity did not always appear in phase with the PS II oscillations in wheat. In most of the cases investigated, the activity peaks of PS I seemed to alternate with those of PS II. This point is suggestive of the possibility that the chloroplast membranes have an ability to regulate the electron transport and perhaps also the energy distribution between the two photosystems during the day by means of modulating the capacity of each of the photosystems such that optimal electron transport is maintained. Analysis of the ratios of PS II activity (O_2 evolution) to that of PS I activity (O_2 uptake) exhibited periodic oscillations (figure 11) which closely corresponded to the rhythm in PS II activity in the respective conditions. Furthermore, the rhythmicity seen in the PS II to PS I ratios represents a closely comparable inverted image of the PS I rhythm. These observations suggest that the PS II oscillations are out-of-phase with PS I oscillations indicating a probable strategy to regulate electron transport and may also be linked to the distribution of absorbed energy between the two photosystems.

In the presence of photophosphorylation uncouplers such as gramicidin-S, the rhythms in electron transport activity persisted (figures 2–10). Similar results were obtained with FCCP and NH_4Cl (data not shown). These results suggest that the observed rhythmicity did not arise simply due to an unequal degree of coupling capacity of the chloroplasts sampled at different parts of day, instead it appears to be an intrinsic time regulated modulation of the electron transport chain. In most of the cases, the oscillations seen in the presence of uncouplers were in phase with those measured without the chemicals. However, there was a slight difference in the pattern of oscillations in LD and LD entrained LL grown plants in that they were not exactly in phase with the basal activity rhythms. Unlike our observations, Lonergan (1981) could not observe any distinct rhythmicity in the legume chloroplasts in the absence of uncouplers but however, a distinct pattern of oscillations emerged upon the inclusion of gramicidin or NH_4Cl in the reaction medium. This observation also suggests that it is the electron transport that fluctuates rhythmically.

The important point we wish to make here is that the chloroplast membranes possess a time triggered ability to respond differentially to the chemical uncouplers. Gramicidin mediated enhancement in electron transport activity was more in the light phase, particularly when the basal activity was low suggesting a higher degree of coupling between electron transport and photophosphorylation during the light phase (figures 2–10). The same was true with NH_4Cl and FCCP treatment (data not shown). The coupling between the electron transport and photophosphorylation in chloroplasts undergoes rhythmic variation. It appears that the thylakoid membranes experience some sort of rhythmic coupling and uncoupling *in situ*, which could have resulted from corresponding changes in the thylakoid membrane conformation. Prezelin and Sweeney (1977) have suggested reversible conformational changes in *Gonyaulax* thylakoid membranes. Rhythmic changes in the chloroplast ultrastructure such as the degree of appression (Vanden Driessche and Hars 1972) and thylakoid orientation (Herman and Sweeney 1975) have been previously reported indicating that these changes may be linked to the rhythms in various thylakoid functions.

Acknowledgement

This work was supported by ICAR-USDA grant (FG-IN-575; IN-SEA-170). One of the authors (SAS) thanks the Council of Scientific and Industrial Research, New Delhi for a fellowship.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 3, June 1988, pp. 175–181.
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Rhythmic oscillations in wheat chloroplast photochemical activity. II. Further characterization of the rhythm in photosystem II photoelectron transport activity

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MS received 6 July 1987

Abstract. Chloroplasts isolated at defined times from the primary leaves of wheat seedlings grown under continuous light as well as 12 h light-dark cycles exhibited endogenous oscillations in photosystem II activity monitored as photoreduction of 2,6-dichlorophenol indophenol with water as electron donor. The 12 h rhythmicity in photoreduction of 2,6-dichlorophenol indophenol resembled the oscillation pattern in photosystem II supported O_2 evolution. Room temperature chlorophyll *a* fluorescence intensity ratios (F_{+DCMU}/F_{-DCMU}) yielded a rhythmicity closely comparable to that of 2,6-dichlorophenol indophenol photoreduction rhythm. Photosystem II mediated photoreduction of 2,6-dichlorophenol indophenol monitored with the exogenous electron donor hydroxylamine which bypasses the electron donation by water also exhibited similar 12 h oscillations. These results suggest that the O_2 evolving complex does not entirely contribute to the origin of the rhythmicity in photosystem II. Instead, periodic changes in the reaction centre-antenna complexes may represent the rate limiting step for the generation of the time triggered rhythms in chloroplast photochemical activity. It is postulated that a temporal reversible control over the functional coupling and uncoupling between the reaction centres and antenna complexes or temporal changes in active and inactive reaction centres may induce the periodic oscillations in PS II activity.

Keywords. Chl *a* fluorescence; hydroxylamine; photosystem II; rhythmicity in chloroplast activity; wheat.

1. Introduction

If the rhythmic oscillations observed in the photosynthetic capacity of various plant systems are indeed restricted to the light reactions (Lonergan 1984), then the question arises as to where the site of such a control in the electron transport chain is located. A number of attempts have been made to establish this point by investigating the temporal pattern of partial electron transport activities in both algae and higher plants. In the chloroplasts of *Euglena gracilis* (Lonergan and Sargent 1979) and *Pisum sativum* (Lonergan 1981) isolated periodically, both photosystem I (PS I) and photosystem II (PS II) mediated electron transport activities remained more or less constant throughout the day. However, using cell free extracts of the green alga *Bryopsis maxima*, Okada and Horie (1979) showed that the PS II activity measured as O_2 evolution supported by benzoquinone or as 2,6-dichlorophenol indophenol (DCPIP) photoreduction oscillated rhythmically like the photosynthetic O_2 evolution in whole cells reported earlier (Okada *et al* 1978). Similarly, in the case of cell free extracts of *Gonyaulax polyedra*, Samuelsson *et al* (1983) also demonstrated a rhythm in PS II supported O_2 evolution monitored as electron transport from H_2O to diaminodurene (DAD) plus FeCN but not in PS I mediated photochemical activity. These authors have therefore suggested that the rhythmicity observed in PS II can account for the circadian oscillation in intact cells.

Wheat chloroplasts exhibit distinct oscillations in their PS II as well as PS I supported electron transport activities with 12 to 16 h periodicity (Sayeed and Mohanty 1988). This observation along with the earlier reports (Okada and Horie 1979; Samuelsson *et al* 1983), indicates that the time-dependent changes in the partial electron transport activities are indeed the constituent parts of the whole chain electron transport or whole cell O_2 evolution rhythms. In this communication, we report the presence of a distinct rhythm pattern of about 12 h periodicity in room temperature chlorophyll (Chl) *a* fluorescence yield and in PS II catalyzed DCPIP photoreduction in the absence and presence of the exogenous electron donor hydroxylamine. These findings suggest that one of the possible sites of generation of the rhythm in photochemical activity is localized in the electron transport chain of PS II.

2. Materials and methods

2.1 *Growth conditions and chloroplast isolation*

Healthy seeds of *Triticum aestivum* L. cv. Kalyansona were germinated on 3 layers of moist germination paper in petri dishes and grown under continuous fluorescent illumination ($\simeq$ 20 W m^{-2}) or under 12 h light-dark cycles as described in the previous report (Sayeed and Mohanty 1988). Periodic isolation of the chloroplasts from about 30 primary leaves and estimation of Chl content were also carried out as outlined before (Sayeed and Mohanty 1988). Measurements of photochemical activity or relative room temperature fluorescence intensity were started at the beginning of a photoperiod which coincided with the sunrise time in the standard clock and marked as zero h.

2.2 *PS II assay*

Photoreduction of DCPIP was monitored spectrophotometrically at 590 nm with water or added hydroxylamine (NH_2OH) as electron donor to PS II using a Hitachi 557 spectrophotometer in the split beam mode. A 250 W actinic light source (GPV 035–20, Takasago, Japan) was used to illuminate the sample cuvette. The actinic beam was filtered using a CS 2–58 Corning red glass filter which provided $\simeq$ 400 W m^{-2} irradiance at the surface of the cuvette. The photomultiplier was shielded from the exciting light by a SZS–23 blue Machinostroeniya (USSR) glass filter. Irradiance was measured using a YSI model 65 A radiometer (YSI, Ohio, USA). The mixture for DCPIP Hill reaction contained 50 mM MES buffer (pH 6·5), 5 mM $MgCl_2$, 10 mM KCl, 20 μM DCPIP and, when present, 10 mM freshly prepared NH_2OH (pH 7). Chloroplasts equivalent to 15–17 μg Chl were taken for each assay.

2.3 *Measurement of Chl a fluorescence*

The relative enhancement in room temperature fluorescence yield at 680 nm upon addition of DCMU was recorded at specified times using a Perkin-Elmer LS-5 luminescence spectrophotometer. Chloroplasts equivalent to 8 μg Chl suspended in 3 ml of reaction mixture (same as in PS II assay, pH 6·5) were excited at 436 nm. DCMU was added to a concentration of 5 μM to the sample cuvette directly after completing the measurements without the herbicide. Five nm slit width was selected for both excitation and emission monochromators. Fluorescence yield was recorded 1 min after the excitation light was put on.

3. Results and discussion

3.1 *Rhythm in Chl a fluorescence*

Since the Chl *a* fluorescence intensity at 680–685 nm is a measure of PS II photochemistry and is sensitive to the electron flow through the reaction centres of PS II (Papageorgiou 1975), it provides an additional method to monitor PS II activity. We have measured the relative intensity of Chl *a* fluorescence at 680 nm in the presence (F_{+DCMU}) and absence of 5 μM DCMU (F_{-DCMU}). Addition of DCMU to the chloroplasts closes all the PS II traps and raises the fluorescence intensity to the maximum level. The data expressed as the ratios F_{+DCMU}/F_{-DCMU} as a function of time of day (figure 1) exhibited a distinct pattern of oscillations with a 12 h

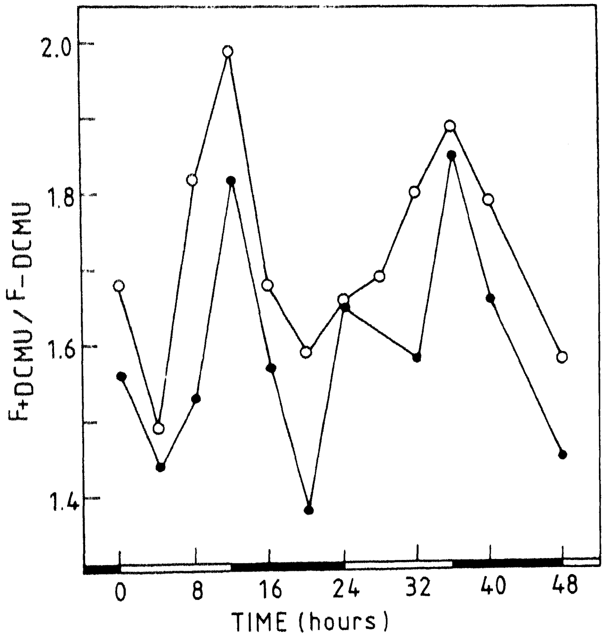

Figure 1. Rhythm in DCMU induced enhancement in Chl *a* fluorescence yield in chloroplasts isolated from the primary leaves of LL (○) and LD (●) grown wheat seedlings. Relative fluorescence emission intensity at room temperature monitored at 680 nm in the absence and presence of 5 μm DCMU is expressed as the ratio F_{+DCMU}/F_{-DCMU}. Points represent averaged data from two experiments. Dark period is represented by solid horizontal bars and 0 h indicates sunrise time in the standard clock. 5 nm slit was selected for both the excitation (436 nm) and emission monochromators. The emission intensity was recorded 1 min after the excitation light was put on.

periodicity in both the LD and LL plants. The rhythm pattern in Chl *a* fluorescence intensity is comparable to that in PS II mediated O_2 evolution (Sayeed and Mohanty 1988). However, the first peak in F_{+DCMU}/F_{-DCMU} appeared at the beginning of the cycle (i.e. zero h) and the first peak of the second cycle was not prominent in this case. There was also a variation of about 4 h in the peak times between the rhythms in Chl *a* fluorescence yield and PS II mediated O_2 evolution.

Maximal values of F_{+DCMU}/F_{-DCMU} indicated that higher rates of electron flow occurred in the light period than during the dark period. This also correlated positively with the DCPIP photoreduction rhythm. The maximum-to-minimum variation in F_{+DCMU}/F_{-DCMU} was of the order of about 1·3 fold. Prezelin and Sweeney (1977) also expressed their data on room temperature Chl *a* fluorescence in *Gonyaulax* cells as F_M/F_{+DCMU} ratios. This expression yielded higher values of the ratio at 180° out-of-phase with the peak values of whole cell O_2 evolution and the peak values indicated lower rates of electron transport. The amplitude in *Gonyaulax* room temperature fluorescence rhythm was also about 1·3 fold.

3.2 *Rhythm in photosystem II hill activity*

Besides the report of Okada and Horie (1979) on the rhythm in PS II activity of *Bryopsis* measured as DCPIP photoreduction as well as benzoquinone supported O_2 evolution and the report on *Gonyaulax* PS II activity rhythm measured as DAD + FeCN supported O_2 evolution (Samuelsson *et al* 1983), other efforts to study PS II hill activity monitored as DCPIP photoreduction in higher plants (Lonergan 1981) have not yielded positive results. It was therefore, of interest to check if the rhythm in PS II supported O_2 evolution with PD_{ox} + FeCN described earlier (Sayeed and Mohanty 1988) could be monitored by the photoreduction of the Hill oxidant DCPIP as well.

Chloroplasts isolated from the primary leaves of wheat seedlings grown under continuous light of 12 h LD cycles exhibited oscillations in the photoreduction of DCPIP (figure 2). Both LL and LD plants showed a rhythm of about 12 h periodicity with a 2–4 fold difference between minimum and maximum values. This oscillation pattern was comparable to the rhythm in room temperature Chl *a* fluorescence yield (figure 1) and to that in PS II supported O_2 evolution (Sayeed and Mohanty 1988) where the LL plants exhibited approximately 12 h periodicity. However, the exact peak times were also not always the same in DCPIP photoreduction and PD_{ox} + FeCN supported O_2 evolution activity. The first peak in DCPIP photoreduction always appeared at the beginning of the experiment at zero h as observed in the case of room temperature fluorescence rhythm (figure 1). The period length in the case of LD and LL between the first two peaks was 12 h, whereas the second to third peak period was about 16 h. The period length between the third and fourth peak was 8 h. However, in the subsequent cycle the period was 12–16 h in the case of LL (figure 2). These peak times differed by about 4 h from that observed in the PS II mediated O_2 evolution rhythm in LL plants. Maximal rates of DCPIP photoreduction were obtained at the beginning and end of the light periods in LD plants. The same period constituted a subjective day-time for the LL plants.

In order to ascertain if the oxygen evolving complex (OEC) is responsible for the rhythmic changes in PS II mediated electron transport activity, PS II photochemical activity was measured with an exogenous donor that bypasses the water oxidation

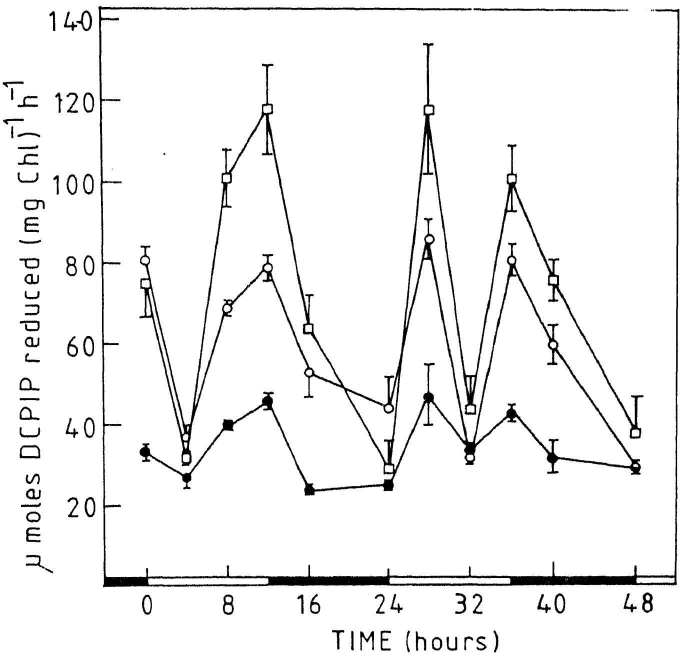

Figure 2. Rhythm in photosystem II DCPIP photoreduction activity in isolated chloroplasts of wheat primary leaves sampled at different times of day from continuous light grown (○) and light-dark grown (□) seedlings. NH_2OH supported DCPIP photoreduction (●) in chloroplasts isolated from LL grown seedlings was also monitored during the same time intervals. Sunrise time in the standard clock was taken as 0 h of the measurements and the night time is represented by solid horizontal bars. Values represent the mean of 12 and 9 replicates respectively for H_2O to DCPIP and NH_2OH to DCPIP measurements.

system. A number of artificial electron donors capable of feeding electrons to PS II without the involvement of OEC, such as 1,5-diphenyl carbazide (DPC), hydroxylamine (NH_2OH) and Mn^{2+}, are available (Trebst 1974). Since DPC can donate electrons to PS II only in the absence of OEC activity, the OEC has to be inactivated by specific treatments such as high temperature or washing with Tris buffer. These treatment procedures are long and time consuming and moreover are difficult to perform routinely after short intervals of time. Therefore, we chose to use the exogenous electron donor NH_2OH which at high concentration directly feeds electrons to the reaction centre of PS II bypassing the donation of electrons by H_2O (Cheniae and Martin 1971; Mohanty *et al* 1971).

NH_2OH supported DCPIP photoreduction showed a rhythmic pattern very similar to that of the H_2O to DCPIP photoreduction reaction (figure 2). The minimum to maximum variation in the oscillation ranged from 1·5–2-fold in LL plants. Even though the extent of variation in the NH_2OH supported DCPIP photoreduction was small, the oscillations were distinct with period and phase characteristics closely comparable to the rhythm in H_2O to DCPIP activity (figure 2). This observation suggests that the rate limiting steps responsible for the origin of the oscillations are not solely linked to OEC but may be associated with the PS II segment of the electron transport chain.

The present study has revealed distinct rhythmicity in the photoreduction of the stable primary electron acceptor Q of PS II, the redox state of which alters the fluorescence yield (figure 1). PS II activity, assayed as DCPIP photoreduction with water and with NH_2OH substituting H_2O as electron donor (figure 2), also oscillated. The rhythmicity observed in NH_2OH supported DCPIP photoreduction further suggests that the oscillation although small in amplitude, is localized in the PS II segment of the electron transport chain. As the PS II photochemical activity without the participation of OEC still exhibited an oscillation pattern similar to the one seen in H_2O to DCPIP photoreduction, we conclude that the OEC may contribute to the rate limiting step partly, but does not entirely constitute such a step.

The other suggestion that the reducing side of the PS II electron transport chain involving the plastoquinone (PQ) pool may have a role in the rhythm generation has been discounted by the reports of Lonergan (1981) and Samuelsson *et al* (1983). Both these reports have shown that the electron transport measurements involving the PQ pool did not oscillate rhythmically. However, faint oscillations were noticed in PS I activity measurements involving the PQ pool. This may be because of the lack of sensitivity of the assaying system suggesting the possibility of an oscillation of a small magnitude in PQ reoxidation reactions.

Our results strongly suggest that the step responsible for the generation of the rhythms in wheat chloroplast photochemical activity is restricted to the PS II segment of the electron transport chain. Temporal changes resulting in such oscillations may involve time triggered activation/inactivation of PS II reaction centres or changes in the antenna complexes.

Acknowledgements

This work was supported by ICAR-USDA grant (FG-IN-575; IN-SEA-170) to PM. One of the authors (SAS) thanks the Council of Scientific and Industrial Research, New Delhi for a fellowship.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 3, June 1988, pp. 183–189.
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Seed protein electrophoresis in six species and two F_1 s of *Cicer*

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MS received 1 May 1987; revised 21 April 1988

Abstract. The water soluble fraction of the seed protein of 6 *Cicer* species and of the two F_1 hybrids of *Cicer arietinum* and *Cicer reticulatum* was analyzed by disc electrophoresis. Each species and F_1 of *Cicer* had its own pattern and also a different number of bands. In general banding pattern revealed variation in number and intensity of bands in all the species and F_1 s. A little difference was in the protein profile of the two strains, NEWC-21 and Jm-2106 of *Cicer reticulatum*. Common and kabuli types of *Cicer arietinum* were found to possess 7 bands but one band in each type was at different R_f. The bands in *Cicer arietinum* had homologues in other wild species and none of the wild species possessed all 7 *Cicer arietinum* bands. All the species, except *Cicer arietinum* and *Cicer judaicum*, possessed some typical bands in their protein profiles in the sense that no one was found at that particular R_f in other species. As regards the similarity indices between hybrids and their respective parents it was found that each cross showed more than 50% homology with their parents. On the basis of seed protein profile, *Cicer reticulatum* seemed to be the most suitable for wild progenitor of *Cicer arietinum*. The difference between the profiles of *Cicer judaicum* and *Cicer pinnatifidum* supported the idea that they are indeed two separate species.

Keywords. Seed; protein; electrophoresis; *Cicer*.

1. Introduction

The seed protein profile is apparently a typical and diagonastic trait for plant species. The seed protein can not only serve as an additional tool in taxonomic studies but might also be helpful in determining and evaluating species relationship among the plant species. It's usefulness has been elaborated in biosystemic studies and the technique has been employed in more than 45 different genera (Ladizinsky and Hymowitz 1979). The disc electrophoresis technique offers the advantages of speed, simplicity, high resolution and great sensitivity. Several modifications have been introduced yielding a method which is simple and rapid, and which possesses excellent resolving power for all basic proteins (Shepherd and Gurley 1966). Larsen (1967) analyzed seed protein in 61 soybean varieties, *Glycine max* (L.) Merrill, by disc gel electrophoresis and was able to classify the varieties into two distinct groups based on two components identified by stained proteins in polyacrylamide gels. In other crops such as wheat (Johnson *et al* 1967), barley (McDaniel 1970) and cotton (Johnson and Thein 1970), species relationship revealed by conventional method and have been confirmed according to their seed protein profiles. Electrophoretic patterns of soluble seed protein have been used as genetic marker in many biosynthetic studies (Johnson 1969, 1972a, b; Yadav *et al* 1979; Jope and Jana 1980). In *Cicer*, where information on species relationship deduced from meiotic behaviour and fertility of the interspecific hybrid (Ladizinsky and Adler 1976a) is not available due to difficulty in crossing the small cleistogamous flowers, the seed protein which reflect the genetic constitution of various species might provide us with some clues (Ladizinsky and Adler 1976b). The present study on different species and F_1s of *Cicer* was undertaken to identify them on the basis of soluble seed protein banding pattern and also to find out the genetic diversity and affinity among different species based on similarity index.

2. Materials and methods

A total of 8 varieties or strains of different species of *Cicer*, viz., H-208 (common type) and ICC-8923 (kabuli type) of *C. arietinum*, Jm-2103 of *C. bijugum*, SL-157 of *C. cuneatum*, Jm-185 of *C. judaicum*, Jm-188 of *C. pinnatifidum*, NEWC-21 and Jm-2106 of *C. reticulatum* were studied for their protein banding pattern. Along with these species F_1 of *C. arietinum* (H-208) × *C. reticulatum* (NEWC-21) and F_1 of *C. arietinum* (ICC-8923) × *C. reticulatum* (Jm-2106) were also studied electrophoretically.

Disc gel electrophoresis (Davis 1964) was adopted to separate the total protein [extracted in 0·1 M Tris HCl buffer, pH 8, and estimated by the method of Lowry (1951) using bovine serum albumin as standard] from the seed. The gels were stained in imido black and destained in 7% acetic acid to remove the unbound dye in order to visualize the protein bands. Sample was layered on 7·5% running gel and electrophoresis was conducted at 3 mA current per gel column. R_f value was calculated for all visible bands and similarity index was calculated according to the method of Sheen (1972).

3. Results and discussion

A total of 30 bands were observed and these bands were numbered with increasing magnitude of R_f values. The types of bands were decided on the basis of their colour intensity, like thin and distinct, diffuse and dense. The protein banding pattern of all the species and F_1s are presented in figure 1. Each species of *Cicer* and F_1s had its own pattern and also a different number of bands. The number and kind of bands in different species and F_1s are given in table 1. In general protein banding pattern revealed variation in number and intensity of bands in all the 6 species and F_1s of *Cicer*. However, little difference was in the protein profile of the two strains NEWC-21 and Jm-2106 of *C. reticulatum*, where it was composed of 8 bands in both the strains but one dense band in each case was at different R_f (0·63 and 0·69 respectively) value. Similarly common and kabuli types of *C. arietinum* were found to possess 7 bands but one band in each type was found at different R_f (0·97 and 0·30 respectively) value. They were not also homologue in the sense that the band in common type was diffuse while the other band in kabuli type was thin and distinct. This was a constant and conservative character in both the types. Ladizinsky and Adler (1976b) found the same basic seed protein profile composed of 7 bands in 88 cultivars of *C. arietinum* except two cultivars where the band at R_f 0·61 split into two close but distinct bands. The per cent similarity index among the different species are given in table 2. In the present study similarity of the protein profile between these two types was 71·43%. Common and kabuli types of *C. arietinum* are treated as the same species. However, Dixit (1932) suggested to place kabuli type in the species to be named *C. kabulium* in the section Arietaria of the genus *Cicer* distinguishing it from

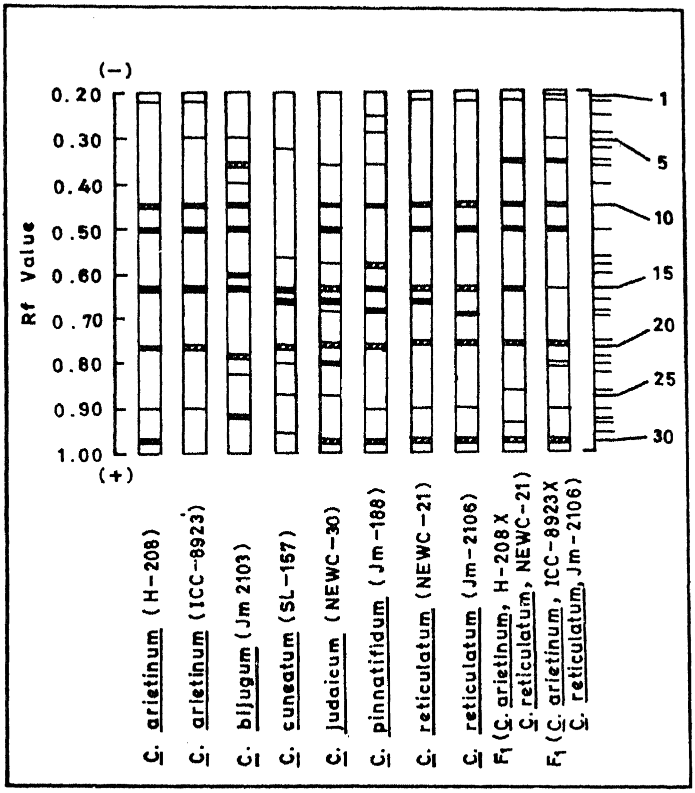

Figure 1. Protein banding patterns in different species and F_1s of *Cicer*.

common type by its large white flowers and large white seeds, and also by chromosome number (2n = 16) as he found 2n = 14 in common type. But it is several times established that both the types have same number of chromosomes (2n = 16).

The bands in *C. arietinum* had homologues in other wild species examined in the present study. However, none of these profiles possessed all 7 *C. arietinum* bands. The profile of *C. bijugum* was of 10 bands, 3 of which had homologues with those of common type *C. arietinum* and 4 of which had homologues with those of kabuli type *C. arietinum*, and their similarity indices were 35·29 and 44·45% respectively. Although *C. bijugum* is more close to the common type than the kabuli type from morphological point of view but that similarity did not reflect in the protein profile. *C. bijugum* showed more close association with kabuli type. Nine bands in *C. bijugum* were reported by Ladizinsky and Adler (1976b) of which 4 had homologues in *C. arietinum* profile. *C. bijugum* showed very poor relation in the protein profile of *C. cuneatum*, *C. judaicum* and *C. pinnatifidum*, where *C. bijugum* had only one homologue in the profile of these 3 species with 11·11, 9·52 and 10% similarity. However, Ladizinsky and Adler (1976b) found 4 and 2 bands of *C. bijugum* showing similarity in the protein profile of *C. judaicum* and *C. pinnatifidum* respectively. The morphological characters of these 4 species display tremendous variation particularly in the shape, size and colour of seeds, number of leaflets, photoperiodical response etc. Similarity indices in the protein profile between *C. bijugum* and *C. reti-*

Table 1. Number and kind of bands in some species of *Cicer* and F_1s.

Species/ F_1s	Total number of bands	Kind of bands Thin and distinct band	Diffuse band	Dense band
C. arietinum H-208	7	2	3	2
C. arietinum ICC-8923	7	3	2	2
C. bijugum Jm-2103	10	3	5	2
C. cuneatum SL-157	8	5	1	2
C. judaicum NEWC-30	11	4	4	3
C. pinnatifidum Jm-188	10	4	3	3
C. reticulatum NEWC-21	8	2	4	2
C. reticulatum Jm-2106	8	2	4	2
F_1 (*C. arietinum*, H-208 × *C. reticulatum*, NEWC-21)	9	3	4	2
F_1 (*C. arietinum*, ICC-8923 × *C. reticulatum*, Jm-2106)	11	5	5	1

Table 2. Estimation of per cent similarity indices for protein bands in some *Cicer* species.

	C. arietinum ICC-8923	*C. bijugum* Jm-2103	*C. cuneatum* SL-157	*C. judaicum* NEWC-30	*C. pinnatifidum* Jm-188	*C. reticulatum* NEWC-21	*C. reticulatum* Jm-2106
C. arietinum H-208	71·43	35·29	26·67	22·22	35·29	66·67	66·67
C. arietinum ICC-8923		44·45	26·67	11·11	35·29	53·33	53·33
C. bijugum Jm-2103			11·11	9·52	10·00	22·22	22·22
C. cuneatum SL-157				21·05	22·22	12·50	12·50
m)					28·57	52·63	42·10
idum						22·22	22·22
							87·50

ls of *C. bijugum* had homologues in *C. reticulatum* vo species are somewhat closer than *C. cuneatum*, *C.* nd this similarity, however, reflected a little in the *n* is the only species which shows climbing habit and and differs apparently from other annual species of

Cicer. The seed protein profile of *C. cuneatum* was composed of 8 bands, two of which had homologues in *C. arietinum* (both common and kabuli type) profile. Ladizinsky and Adler (1976b) reported 7 bands in *C. cuneatum* two of which had homologues in *C. arietinum* profile. With *C. judaicum*, *C. pinnatifidum* and *C. reticulatum*, *C. cuneatum* showed 21·05, 22·23 and 12·50% similarity in the protein profile. Out of 8 bands, two had homologues in *C. judaicum* and *C. pinnatifidum*, but only one had homologue in *C. reticulatum*. *C. judaicum* possessed 11 bands, 3 of which had homologues in the protein profile of *C. pinnatifidum*. The protein profile of *C. pinnatifidum* was composed of 10 bands. However, Ladizinsky and Adler (1976b) found 9 and 7 bands for these two species respectively. In the present study these two species showed 28·57% similarity indices in their protein profile. Morphologically they differ from each other in leaflet shape, number of leaflets and seed size. Maesen (1972) following more detailed morphological comparisons decided to keep them as two separate species, since various botanists considered them as variants of the same species. Ladizinsky and Adler (1976b) strongly supported this conclusion by the difference in their seed protein profile. They further mentioned that of the total 16 bands in the profile of these two species, only 3 pairs could be matched. The present investigation has confirmed the homology of 3 pairs of bands out of 18. Thus the conclusion made by Maesen (1972) is strongly supported by the difference in their seed protein profile. On the other hand *C. judaicum* showed more similarity in the protein profile of *C. reticulatum* than that of *C. bijugum*, *C. cuneatum* and *C. pinnatifidum*. However, *C. judaicum* showed different similarity indices with two strains of *C. reticulatum*, i.e., with NEWC-21 similarity index was 52·63% and with Jm-2106 it was 42·10%. Out of 11 bands in *C. judaicum*, 5 bands had homologues in the protein profile of NEWC-21 and 4 had homologues in that of Jm-2106. Morphologically *C. judaicum* and *C. reticulatum* display tremendous variation. *C. pinnatifidum* had two homologues in the protein profile of both the strains of *C. reticulatum* and similarity index was only 22·22%. This species also differs tremendously in morphological characteristics from *C. reticulatum*. The similarity index in protein profile of NEWC-21 and Jm-2106 was 87·50%. NEWC-21 had 6 homologues in the protein profile of Jm-2106. On the other hand both the strains of *C. reticulatum* had 5 homologues in the protein profile of common type *C. arietinum* with 66·67% similarity. Out of 8 bands in both the strains of *C. reticulatum* 4 bands had homologues in the protein profile of kabuli type *C. arietinum* and similarity index between them was 53·33%. Morphologically, *C. arietinum* (common type) and *C. reticulatum* are very close than any other species examined in the present study. They differ from each other mainly in the seed coat structure. *C. arietinum* (kabuli type) differs from *C. reticulatum* mainly by flower colour and the shape, size and colour of seeds. Ladizinsky and Adler (1976b) reported 6 bands in the protein profile of *C. reticulatum* and all of which had homologues in *C. arietinum* and it lacked only one band. Except *C. arietinum* and *C. judaicum*, all the species possessed some typical bands in their protein profile in the sense that no one was found at that particular R_f in other species. *C. bijugum* had two thin and distinct bands at R_fs 0·40 and 0·82. *C. cuneatum* had 3 thin and distinct bands at R_fs 0·32, 0·56 and 0·95. *C. pinnatifidum* had a single thin and distinct band at R_f 0·25. Ladizinsky and Adler (1976b) found two such typical bands at R_f 0·64 and 0·69 in the protein profile of *C. pinnatifidum*. *C. reticulatum* (Jm-2106) had also one dense band at R_f 0·69 in the present investigation. It was constant in species specific character and therefore it may be considered as conservative characteristic in their protein profile.

Table 3. Estimates of per cent similarity indices between F_1s and parents.

F_1 of crosses	Parents	Similarity index (%)
H-208 × NEWC-21	H-208	62·50
	NEWC-21	58·50
ICC-8923 × Jm-2106	ICC-8923	55·56
	Jm-2106	63·16

As regards the similarity indices between hybrids and their respective parents (table 3), it was found that each cross showed more than 50% homology with their respective parents. This proves the greater diversity of the hybrids over pure lines (Singh *et al* 1983). Protein profile in the hybrid of *C. arietinum* (H-208) × *C. reticulatum* (NEWC-21) was composed of 9 bands, 5 of which had homologues in the protein profile of both the parents. The similarity indices of the hybrid with its maternal and paternal parent were 62·50 and 58·50% respectively. Thus it appeared that loci controlling the bands in maternal parent were dominant. The hybrid of *C. arietinum* (ICC-8923) × *C. reticulatum* (Jm-2106) had 11 bands, 5 of which had homologues in the protein profile of maternal parent and 6 had homologues in that of paternal parent with 55·56 and 63·16% similarity indices respectively. Thus it appeared that loci controlling the bands in paternal parent were dominant. Three bands (R_fs 0·36, 0·86 and 0·93) present in the hybrid of *C. arietinum* (H-208) × *C. reticulatum* (NEWC-21) were absent in both the parents, showing complementary nature of their inheritance. Similar bands (R_fs 0·21, 0·36, 0·63 and 0·80) of the hybrids of *C. arietinum* (ICC-8923) × *C. reticulatum* (Jm-2106) were absent in both the parents. This discrepancy in effect at band level suggested that reliable inferences regarding the inheritance of individual bands can not be drawn unless individual F_2 seeds in all the possible crosses are analyzed electrophoretically (Singh *et al* 1983).

On the basis of seed protein profile of 6 species of *Cicer* and F_1s, it appeared that *C. reticulatum* was close to the cultivated species *C. arietinum* than any other wild species examined in the present study. The next most interesting point in the evolution of annual species was the origin of cultivated species. The seed protein profile of this species was found as constant conservative character which indicated monophyletic origin. Ladizinsky and Adler (1976) stated that if the seed protein profile of the present day *C. arietinum* cultivars was virtually with that of the type domesticated thousands of years ago, it was probably also similar to that of its wild progenitor and hence the latter should be detectable among the wild species. In the present study, none of the wild species had identical profile to that of the cultivated species and from this point of view none could be regarded as the immediate progenitor of cultivated species. On the basis of similarity indices in the protein profile of *C. arietinum* and *C. reticulatum* and also of their F_1s, *C. reticulatum* might be the wild progenitor of *C. arietinum* or its close relative.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 3, June 1988, pp. 191–197.
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Airborne fungal spores at Nagarjunanagar

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MS received 11 May 1987; revised 5 March 1988

Abstract. Airborne fungal flora of Nagarjunanagar was studied by using vertical cylinders for a period of two years, from 1st January 1982 to 31st December 1983 at 12 m height and for one year (during 1982) at 1 m height. Out of 75 fungal spore types identified, only 10–30 types were observed on more than 200 days in a year. *Cladosporium* was the dominant type and contributed more than 25% to the total counts. Aspergilli, *Alternaria*, *Periconia* and *Nigrospora* were the other major spore types. The total airborne fungal spora showed a distinct seasonal peak in winter at 1 m and at 12 m a summer peak was observed during 1982 in addition to the winter peak. Circadian rhythms were recorded for 18 spore types by using rotorod samplers.

Keywords. Vertical cylinder; rotorod sampler; air spora; periodicity.

1. Introduction

The population of microbes, pollen and other biological particles in the atmosphere is referred to as air spora by Gregory (1952) and the fungal spores were found to be a major component of the air spora (Gregory 1973; Lacey 1981). It merits study both in its own right as a natural phenomenon and also because it is a component of the air we breathe. The aerobiological studies conducted in India during the last 35 years showed the existence of a rich fungal spore population at various places. In Andhra Pradesh extensive aero-mycological studies were conducted at Visakhapatnam (Sreeramulu and Ramalingam 1966). Similar studies were undertaken at Nagarjunanagar to provide a comprehensive picture on air spora for this part of the country.

2. Materials and methods

Vertical cylinders of 0·5 cm diameter were exposed daily for a period of two years (1st January 1982 to 31st December 1983) at 12 m height on the top of the Botany Department building. The traps were also operated for a period of one year (during 1982) at 1 m height to know the influence of height on the incidence of airborne fungal spores. The exposed cylinders were prepared for microscopy following the method of Ramalingam (1968). An area of 0·18 cm^2 was scanned from each exposure and the spore counts were converted to a number per sq cm. Rotorod samplers (Perkins 1957) were operated on two selected days of each month with normal weather conditions to study the circadian rhythms of fungal spore types. Samples were taken at 2 h intervals and the counts were converted to a numbers per m^3 of air.

3. Results

The atmosphere was never free from fungal spores on any day of the year at Nagarjunanagar.

3.1 *Fungal spores*

More than 75 types of fungal spores were observed on the spore traps. However, not more than 30 types were recorded on any single day. At 1 m, 7 to 35 spore types were observed with catch range from 17 to 2,813/cm^2 in 1982. At 12 m, 2 to 36 spore types were observed on each day, the catch ranging from 13 to 2,833/cm^2 during 1982 and 1983.

3.2 *Contribution of different fungal spore types to total fungal spora*

The yearly mean concentrations of 37 fungal spore types for which individual counts were taken are given in table 1 together with their per cent contribution to the year's total mycoflora.

At 1 m, Aspergilli appeared on 110 days with an yearly mean of 96·09 per sq cm area of the trap surface, contributing 29·34% to the total fungal spora followed by *Cladosporium* (14·7%), *Alternaria* (9·97%) and *Periconia* (6·7%). The rest of the spore types contributed less than 5% in each case. However, at 12 m, *Cladosporium* was found to dominate over other spore types, contributing 31·8% during 1982 and 28·8% in 1983. Aspergilli occupied the second place with a contribution of 10% to the total fungal spora. The conidia of *Alternaria*, *Periconia* and *Nigrospora* were the other major spore types in that order, contributing more than 5% to the total fungal spora.

3.3 *Seasonal periodicity*

Figure 1 shows day to day changes in the total fungal spore catches observed at 1 m during 1982 and at 12 m during 1982 and 1983. At 1 m level, two seasonal peaks were recorded, one in January and February and another in November. However, at 12 m, higher concentrations were also recorded in June, soon after the commencement of monsoon rains, in addition to the above described peaks.

Figure 2 shows the monthly catches of selected fungal spore types recorded during 1982 at 12 m. The conidia of *Cladosporium* appeared in higher numbers in January and February and also in June. Peak concentrations of Aspergilli were observed in November and December. *Alternaria* spores appeared throughout the year with higher numbers in February. *Periconia* showed seasonal peak in February and minimal catches were recorded in summer period. *Nigrospora* showed seasonal peak in September and minimum during October. The conidia of *Pithomyces* showed seasonal peak in the early part of the year (January–February). The conidia of *Curvularia* were observed almost throughout the year with peaks during August–September period. The conidia of various species of *Drechslera* such as *D. hawaiiensis*, *D. oryzae*, *D. halodes*, *D. rostrata* and *D. longirostrata* were observed on the trap slides and were counted as a single group. Their catches were relatively higher during rainy season. The conidia of *Corynespora* appeared throughout the year with peaks in November and February. *Cercospora* conidia were caught in higher numbers in March and July.

All the basidiospore types were counted as a single group. Their peak incidence was observed in the period January to March, and in the rainy season. Peak inci-

Table 1. Yearly mean concentrations of different fungal spore types and their per cent contributions to the total airborne fungal spora at 1 and 12 m height.

	1 m 1982		12 m 1982		12 m 1983	
Spore type	Yearly mean conc. (no./sq. cm.)	Contribution to total fungal spora (%)	Yearly mean conc. (no./sq. cm.)	Contribution to total fungal spora (%)	Yearly mean conc. (no./sq. cm.)	Contribution to total fungal spora (%)
Alternaria	32·7	9·97	37·0	10·42	29·2	9·66
Ascospores	4·7	1·40	4·4	1·25	5·1	1·69
Aspergilli	96·1	29·34	41·7	11·81	33·2	10·96
Basidiospores	20·4	6·23	13·0	3·65	18·6	6·15
Bunt spores	2·2	0·68	1·0	0·29	1·1	0·38
Cercospora	3·0	0·91	2·0	0·56	2·4	0·78
Cladosporium	48·2	14·73	102·3	31·79	87·1	28·77
Conidiophores	5·7	1·73	11·3	3·19	5·7	1·89
Corynespora	4·9	1·45	6·6	1·89	4·4	1·46
Curvularia	12·5	3·82	9·6	2·73	12·3	4·05
Dendryphiella	0·0	0·01	0·2	0·07	0·2	0·06
Diplodia	1·5	0·45	1·2	0·34	1·5	0·50
Drechslera	12·4	3·78	8·7	2·47	8·9	2·90
Fusarium	1·2	0·37	0·9	0·25	1·0	0·32
Ganoderma	—	—	0·4	0·13	0·6	0·21
Hyphal fragments	15·1	4·61	17·7	5·03	13·5	4·46
Leptosphaeria	0·1	0·04	0·0	0·001	0·1	0·02
Leptosphaerulina	0·2	0·07	0·3	0·18	0·2	0·06
Memnoniella	—	—	—	—	0·3	0·12
Nakataea	—	—	0·0	0·01	0·1	0·04
Nigrospora	7·5	2·27	20·5	5·82	21·7	7·18
Oidium	—	—	0·0	0·01	0·1	0·02
Papularia	1·9	0·58	3·1	0·88	2·4	0·81
Periconia	22·1	6·75	30·0	8·48	27·1	8·96
Pestalotia	0·0	0·002	—	—	0·0	0·01
Phaeotrichoconis	2·0	0·62	1·3	0·36	1·1	0·36
Pithomyces	7·5	2·38	10·1	2·86	6·3	2·10
Pleospora	0·3	0·08	0·9	0·24	0·9	0·29
Spegazzinia	0·4	0·11	0·4	0·11	1·0	0·33
Sporormia	0·9	0·27	0·9	0·26	0·9	0·28
Stemphylium	0·0	0·02	1·1	0·31	0·8	0·27
Teleutospores	0·0	0·01	0·0	0·01	—	—
Tetraploa	1·8	0·54	1·0	0·30	2·0	0·65
Tetracoccosporium	—	—	0·1	0·04	0·0	0·01
Torula	0·9	0·26	0·9	0·25	1·9	0·64
Trichoconis	2·3	0·71	3·0	0·84	2·2	0·74
Urediniospores	14·8	4·53	10·6	3·01	8·1	2·66
Unclassified	2·3	0·70	2·0	0·30	2·3	0·80

dence of urediniospores was observed in February. The ascospores, counted as a heterogenous group, recorded peak in February. The ascospores of *Pleospora* showed seasonal peak in rainy season.

Hyphal fragments were trapped more frequently in 1982 than in 1983. They were caught in higher numbers during January to March with peak in the middle of February.

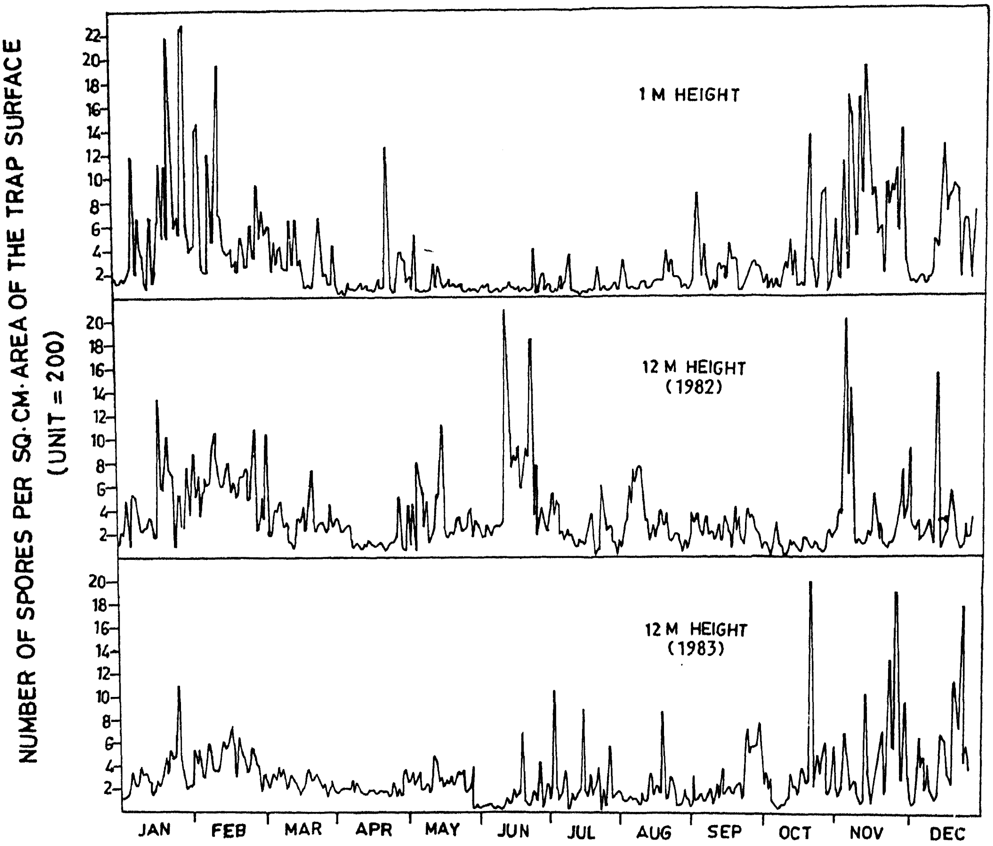

Figure 1. Day to day changes in total airborne fungal spore concentrations estimated per sq cm area of the trap surface.

3.4 *Circadian periodicity*

Circadian rhythms exhibited by 18 fungal spore types, at a height of 1 m, constructed as percentages of the peak arithmetic mean concentration are given in figure 3. The spore types could be classified under the following patterns.

3.4a *Night pattern*: Ascospores, basidiospores of *Ganoderma* showed peak at midnight. Unclassified basidiospores and *Fusarium* showed peaks at 4 am.

3.4b *Post dawn pattern*: The ascospores of *Leptosphaerulina* showed a sharp peak at 8 am.

3.4c *Forenoon pattern*: The conidia of *Cladosporium, Nigrospora, Corynespora, Drechslera, Torula* and *Tetraploa* were placed under this type since they showed peaks at 10 am. A minor peak was observed for *Corynespora* at 2 pm and for *Drechslera* and *Torula* at 4 pm.

3.4d *Midday pattern*: *Alternaria, Curvularia, Trichoconis, Periconia* and hyphal

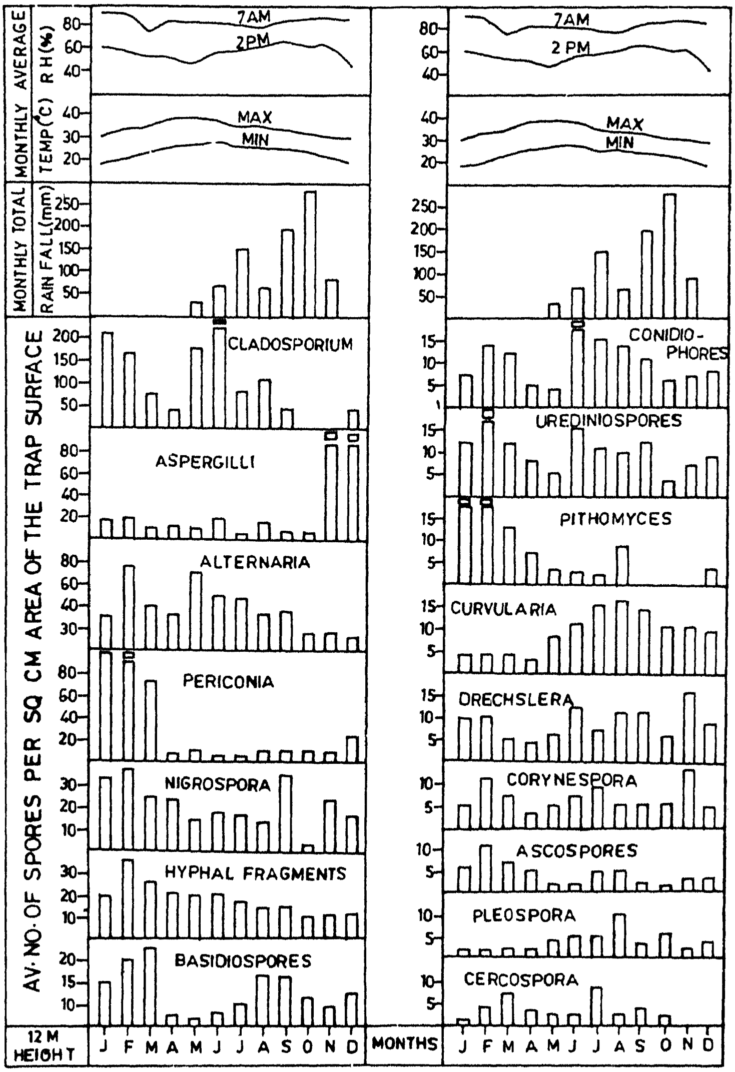

Figure 2. Monthly means of 16 airborne fungal spore types together with monthly means of maximum and minimum temperatures (°C) relative humidity at 7 am and 2 pm (%) and monthly total rainfall (mm) at 12 m height, during 1982.

fragments showed peak at noon. The concentrations of all these types began to build up at 10 am and gradually declined.

3.4e *Afternoon pattern*: The conidia of *Pithomyces* and the ascospores of *Pleospora* showed peaks at 2 and 6 pm respectively.

The circadian rhythms exhibited by the different fungal spore types at 12 m height were similar with those at 1 m height during this study but some differences occurred with respect to the time at which maximum and minimum concentrations were recorded.

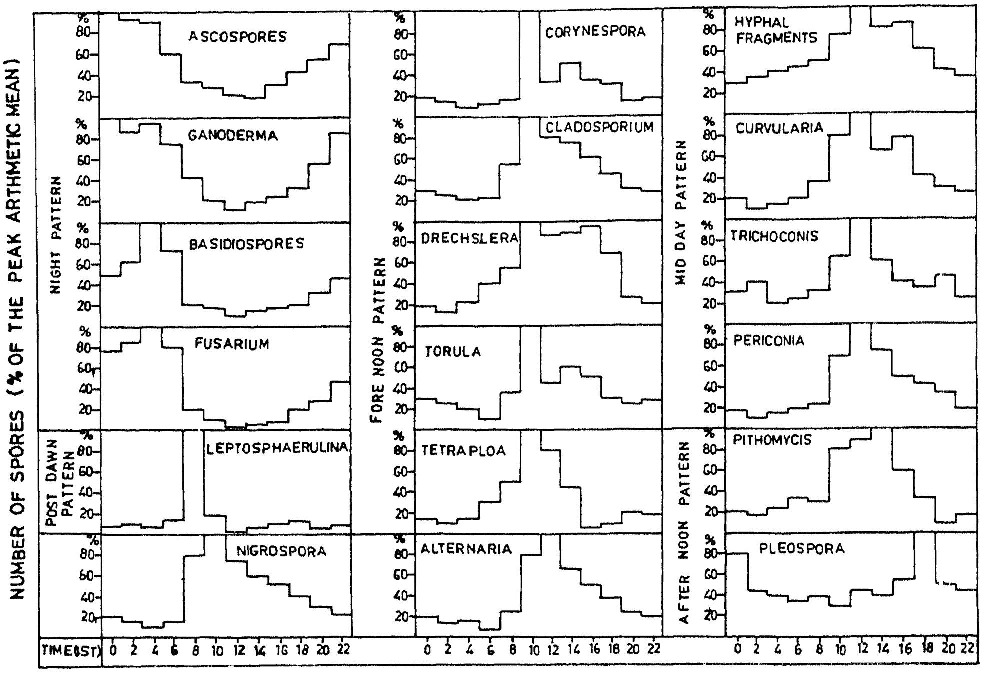

Figure 3. Circadian periodicity patterns of 18 airborne fungal spore types.

4. Discussion

Most aeromycological surveys were conducted by placing the spore traps near ground level (1 or 2 m) or on the top of the building, but not simultaneously at both heights for extended periods as installed in the present investigation. It was observed that the concentration of total fungal spores was slightly higher at 12 m height than at 1 m. A general decline of spore catches with height is reported by Gregory and Hirst (1957) and it is related to the sources of fungal spora from vegetation and ground. Relatively higher concentrations of fungal spores at 12 m is probably due to poor sources from ground level at the trapping sites when compared to that reaching the place from distant sources. Gregory and Hirst (1957) has also reported similar unexpectedly large concentrations on certain days.

The conidia of *Cladosporium*, Aspergilli, *Alternaria*, *Periconia*, *Curvularia* and *Drechslera* were found to be the major contributors for air spora as pointed out by Lacey (1981). Whether in temperate regions or in tropics, spore concentrations were higher towards the end of crop growing season, which could be related to the accumulation of dry matter and saprophytic colonization on them. Most of the conidial types showed peaks in winter months, whereas ascospores and basidiospores were observed in higher numbers during rainy season and this type of seasonal incidence of fungal incidence was also reported by other workers in India (Sreeramulu and Ramalingam 1966; Mallaiah and Rao 1980).

Most of the fungal spore types observed during the present study showed a rhythmic pattern of appearance in relation to the time of the day. That the airborne spore types show a clear circadian rhythm was also reported by various other workers using volumetric spore trapping technique.

Acknowledgements

Thanks are due to Prof. A S Rao, for providing the necessary facilities. One of the authors (PSSR) is thankful to the Council of Scientific and Industrial Research, New Delhi for awarding a fellowship.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 3, June 1988, pp. 199–204.
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Ammonium accumulation and glutamine synthetase activity in C_3 and C_4 plants*

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MS received 29 August 1987; revised 20 April 1988

Abstract. Glutamine synthetase activity was found to be several fold higher in the leaves of a few C_3 as compared to C_4 plants. Differences in NH_4^+ levels were marginal. Methionine sulphoximine inhibition of glutamine synthetase resulted in increase in NH_4^+ levels more in wheat than in sorghum. The decrease in methionine sulphoximine induced accumulation of free NH_4^+ by photorespiratory inhibitors, isonicotinic acid hydrazide and α-hydroxy methane pyridine sulphate was also several fold higher in wheat than in sorghum. Feeding of glycine and glycolate, intermediates of photorespiration, showed the presence of active glycine decarboxylation system in sorghum but the metabolism of glycolate was more efficient in wheat. Higher glutamine synthetase activity in C_3 plants may be a necessary adaptation to a higher photorespiratory NH_4^+ production and explain the similar or higher N losses in C_4 as compared to C_3 plants. These results also support the hypothesis that glutamine synthetase is important in the assimilation of photorespiratory ammonia.

Keywords. Glutamine synthetase; NH_4^+ levels; C_3 and C_4; photorespiratory nitrogen cycle.

1. Introduction

Ammonium ions are products of several metabolic pathways. Apart from nitrate reduction, oxidative decarboxylation of glycine during photorespiration is another reaction leading to production of NH_4^+. While the reaction is though to be of considerable quantitative importance in C_3 plants (Keys *et al* 1978), fluxes of N via this pathway are very low in C_4 (Berger and Fock 1983a,b). Accordingly, there is a possibility of built up of higher NH_4^+ concentration in C_3 than C_4 plants with concomitant gaseous N loss. However, an analysis of a variety of crop and weed species by Stutte and Weiland (1978) revealed that both C_3 and C_4 plants lose similar amount of N. In fact, it has been reported recently (Weiland and Stutte 1985) that volatilization was higher from sorghum (C_4) than from soyabean (C_3). The apparent lack of expression of the large differences of ammonia production in N volatilization generates interest in a comparative study of the metabolism of ammonia in C_3 and C_4 species. A few experiments were, therefore, conducted to compare the accumulation and assimilation of ammonia in C_3 and C_4 leaves. Preliminary findings pertaining to some of these aspects were reported earlier (Maheswari *et al* 1985).

2. Materials and methods

Grains of wheat (*Triticum aestivum* L.) cv. HD 2204: Sorghum (*Sorghum bicolor* L. Moench) Hyb. CSH-1 and CSH-5; chickpea (*Cicer arietinum* L.) cv. BG 274; rice

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(*Oryza sativa* L.) cv. Pusa 221; maize (*Zea mays* L.) Hyb. Ganga 5; pearl millet (*Pennisetum americanum* L K Schum) Hyb. BJ 104 and common millet (*Panicum miliaceum*) Hyb. GPMR 573 obtained from the Genetics Division of the Institute were used.

2.2 *Experiment I*

Seedlings of wheat and sorghum were raised in plastic pots (15 × 15 cm) in sand culture at a temperature of 25°C with 13 h light, 11 h dark and 80% humidity. These were watered with deionized water till the appearance of the first leaf. Subsequently, full Hoagland's solution containing KNO_3 was supplied. Free NH_4^+ content and glutamine synthetase (GS) activity were determined in the third leaf at 15, 25, 35 and 45 days in wheat and 8, 18, 28 and 38 days after emergence in sorghum.

2.3 *Experiment II*

Seedlings of wheat and sorghum were raised as in experiment I and the third leaf of 30 day old plants was used for the feeding experiments.

2.4 *Experiment III*

Seedlings of wheat, chickpea, rice, maize, pearl millet, sorghum and common millet were raised in cement pots (38 × 72 cm) containing soil. The pots were placed in the net house. There were 4 replicates. Free NH_4^+ content and GS activity were analysed in the top two fully expanded leaves of 25 day old plants.

2.5 *Protein content*

Protein in the leaf extracts was estimated following the methods of Lowry *et al* (1951) after precipitation with TCA.

2.6 *Free NH_4^+ content*

Samples were homogenised in 25 mM potassium phosphate buffer (pH 7·3). The homogenate was centrifuged at 12000 *g* for 20 min. Ammonia was diffused from the extract after liberation by sodium tetraborate (pH 10) into traps of 2% boric acid in Warburg flasks. NH_4^+ was determined following the method of Novazamsky *et al* (1974). The absorbance was read at 630 nm.

2.7 *GS activity*

Samples were homogenised with 10 volumes of extraction medium to 1 g leaf tissue. The extraction medium prepared according to Mohanty and Fletcher (1980) with slight modification (Hirel and Gadal 1982) consisted of 100 mM Tris HCl, 10 mM sucrose, 10 mM β-mercaptoethanol, 10 mM KCl, 10 mM dipotassium EDTA and 10 mM $MgCl_2$. The pH was adjusted to 6·8. The homogenate was centrifuged at

15000 *g* for 20 min. The assay procedure involved estimation of ATP dependent formation of γ-glutamyl hydroxamate (Rowe *et al* 1970). The reaction mixture consisted of Tris 100 μmol, pH 8 (pH 7·8 in the case of sorghum); ATP, 10 μmol; sodium glutamate 250 μmol; $MgSO_4$ 100 μmol; cysteine 10 μmol; hydroxylamine hydrochloride 10 μmol and 0·3 ml enzyme extract in a final volume of 3 ml. The reaction was initiated with the addition of $NH_2OH{\cdot}HCl$ which was omitted in the blank. After incubation for 15 min at 37°C, the reaction was terminated by adding 1 ml of ferric chloride (prepared by mixing equal volumes of 10% w/v $FeCl_3{\cdot}6H_2O$ in 0·2 N HCl, 24% TCA and 50% (v/v) HCl). The absorbance of the supernatant was read at 540 nm after removing the pellet by centrifugation at 3,000 *g*.

2.8 *Incubation of leaves*

Third leaves of wheat and sorghum were cut under water and placed vertically in small culture vials containing different solution and incubated in light at radiant flux intensity of 220 μE $m^{-2}S^{-1}$ for 90 min. In all the treatments where methionine sulphoximine (MSX) was used in combination with other compounds, the leaves were first incubated with MSX for 20 min to ensure GS inhibition. At the end of 90 min the leaves were washed, sliced, frozen in liquid nitrogen and stored at 20°C till further analysis. The concentration of various compounds was as follows: MSX, 2·5 mM; INH, 70 mM; α-hydroxy methane pyridine sulphate (α-HPMS), 20 mM; glycine, 15 mM and glycolate, 15 mM.

3. Results

Free NH_4^+ levels (μmol g^{-1} dry wt) in the third leaf blade were significantly higher in sorghum than in wheat at all the stages. Reverse was true for GS activity. The activity was 7-fold higher in wheat than in sorghum (table 1).

Table 2 summarises the extent of photorespiratory ammonia production in wheat versus that in sorghum. MSX inhibition of GS increased the NH_4^+ levels 3 times over the control in wheat while in sorghum the increase was about 1·7 times. Accumulation of NH_4^+ was 2·8 times higher in wheat compared to sorghum when GS

Table 1. Free NH_4^+ content (μmol g^{-1} dry wt) and GS activity (μmol γ-glutamyl hydroxamate formed g^{-1} dry wt 15 min^{-1}) in the third leaf of wheat and sorghum.

Genotype (G)	Stage (days)	NH_4^+	GS
Wheat cv. HD 2204	15	11·4	161·4
	25	14·8	326·1
	35	14·7	460·4
	45	14·3	282·4
Sorghum Hyb. CSH-1	8	14·5	38·1
	18	16·3	51·3
	28	16·7	42·9
	38	20·3	37·1

LSD's for NH_4^+: G = 0·35; S = 0·49; G × S = 0·69.
LSD's for GS: G = 14·08; S = 19·91; G × S = 28·16.

Table 2. Effect of MSX and inhibitors of photorespiratory N cycle on the endogenous NH_4^+ levels in the leaves of wheat and sorghum.

	Genotype	
Treatment*	Wheat	Sorghum
	μmol NH_4^+	
Control	13·8	15·5
MSX	41·5 (100)	25·1 (100)
α-HPMS	16·4	14·3
INH	18·9	15·3
MSX + INH	16·8 (40)	23·1 (91)
MSX + α-HPMS	16·1 (38)	16·8 (66)

*Concentration of various inhibitors are given under Materials and Methods.

Numbers in parentheses represent the accumulation of NH_4^+ relative to those of MSX alone.

Each value is a mean of two individual analysis.

was inhibited. The decline in MSX-induced accumulation of free NH_4^+ by photorespiratory inhibitors, INH and α-HPMS, was also several fold higher in wheat than in sorghum.

When glycine was fed alone there was slight increase in the free NH_4^+ levels but when glycine was fed in combination with MSX, the free NH_4^+ levels increased considerably in both sorghum and wheat. Isonicotinic acid hydrazide (INH), an inhibitor of glycine oxidation, reduced the NH_4^+ levels considerably in both the genotypes. Glycolate, another intermediate and a precursor of glycine in photorespiratory N cycle, increased the free NH_4^+ levels in wheat but did not alter the level in sorghum. However, when glycolate was fed alongwith MSX, NH_4^+ levels enhanced in sorghum and wheat. α-HPMS, an inhibitor of glycolate oxidation, brought down the level of NH_4^+ in both the plants when it was fed with MSX and glycolate (table 3).

In experiment III wherein a few C_3 and C_4 plants were used for study it was observed that GS activity expressed on g^{-1} fresh wt basis is approximately 4-fold higher in C_3 than in C_4 plants. Similar differences were maintained when the activity was examined on g^{-1} dry wt and mg^{-1} protein basis (table 4). In wheat and sorghum, the magnitude of activity was of the same order as observed in experiment I.

4. Discussion

In the present comparative study on NH_4^+ levels it was observed that in sorghum (C_4), its level was marginally higher than in wheat (C_3). In earlier investigations, similar levels of NH_4^+ in a number of C_3 and C_4 plants have been reported (Martin *et al* 1983; Kumar *et al* 1984; Maheshwari *et al* 1985). This is despite the experimental evidence presented on the basis of feeding inhibitors of GS photorespiratory nitrogen cycle in this communication and a number of earlier studies (Martin *et al* 1983; Kumar *et al* 1984; Tournier and Gerster 1984) that ammonia fluxes are manifold higher in C_3 compared to C_4 plants. This apparent discrepancy is explained on the basis of higher

Table 3. Effect of the inhibitors and intermediates of the photorespiratory N cycle on the NH_4^+ accumulation in the leaves of wheat and sorghum.

Treatment*	Genotype Wheat	 Sorghum
	μmol NH_4^+	
Control	13·8	15·5
MSX	41·5	25·1
Glycine	22·2	16·6
MSX + glycine	50·7	31·0
MSX + glycine + INH	32·0	24·0
Glycolate	19·6	14·1
MSX + glycolate	44·7	25·8
MSX + glycolate + α-HPMS	19·2	21·4

*Concentration of various inhibitors and metabolites are given under Materials and Methods. Each value is a mean of two individual analysis.

Table 4. GS activity in some C_3 and C_4 plants.

Genotype	GS activity (μmol γ-glutamyl hydroxomate 15 min^{-1}) g^{-1} fresh wt	 g^{-1} dry wt	 mg^{-1} protein
C_3			
Wheat cv. HD 2204	38·3 ± 0·33	356·2 ± 6·39	8·6 ± 0·22
Chick pea cv. BG 274	45·5 ± 0·26	253·4 ± 1·12	12·6 ± 0·18
Rice cv. Pusa 221	35·7 ± 0·42	393·1 ± 4·11	—
C_4			
Maize Hyb. Ganga-5	11·9 ± 0·52	115·4 ± 5·01	3·0 ± 0·14
Pearl Millet Hyb. BJ 104	14·3 ± 0·20	125·9 ± 1·80	3·4 ± 0·07
Sorghum Hyb. CSH-5	10·1 ± 0·33	59·1 ± 1·81	5·7 ± 0·6
Common millet Hyb. GPMR-573	10·1 ± 0·34	100·6 ± 3·32	1·9 ± 0·06

GS activity in wheat as compared to sorghum and a few other C_3 and C_4 plants examined in the study. It is likely that in C_3 plants the capacity of assimilatory system would quantitatively have to be geared to the rates of photorespiratory ammonia release while in C_4 plants such an adaptive mechanism may not be necessary. The presence of higher GS activity in C_3 group of plants support indirectly the suggestion (Keys *et al* 1978; Martin *et al* 1983) that GS is responsible for the reassimilation of major portion of photorespiratory ammonia.

Recently, Foster and Stutte (1986) in their studies with soyabean (C_3) and palmer amaranth (C_4) obtained a reverse relationship between ammonia loss and GS activity. This confirms our earlier observation on GS activity (Maheswari *et al* 1985) and supports the findings reported in the present communication.

Acknowledgement

Thanks are due to Mr Harish Pandey for help in analysis.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 3, June 1988, pp. 205-208.
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Chemotaxonomic studies on some members of Anacardiaceae

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MS received 10 August 1987; revised 20 April 1988

Abstract. Nineteen taxa belonging to 13 genera of the Anacardiaceae have been screened for leaf flavonoids, phenolic acids, saponins, tannins, alkaloids and iridoids. The family characteristically contains highly hydroxylated compounds like myricetin and gallic acid. It is also rich in proanthocyanidins and flavonols such as quercetin, kaempferol and their methoxylated derivatives. Tannins are common, saponins rare and alkaloids and iridoids are absent. The chemical differences among the various tribes are not very pronounced. However, the tribe Mangiferae does not contain any flavone whereas the tribe Spondieae is comparatively rich in these compounds. The tribes Rhoideae and Semecarpeae are similar in many chemical features. The separate identity of *Chaerospondias* away from *Spondias* is also established in the light of chemical evidences.

Keywords. Anacardiaceae; chemotaxonomy; flavonols.

1. Introduction

The family Anacardiaceae is distinguished from the related families by the presence of intrastaminal disc, resin ducts, unilocular ovary and drupaceous fruits. This family includes a number of economically important plants which yield edible fruits, gums, resins, tan-dyes and wood. It is also known for their toxic long chain aliphatic phenolic compounds such as anacardol, anacardic acid, urushol and hydrourushol; which possess irritant and allergenic properties.

Anacardiaceae are customarily placed in Sapindales alongwith the closely related families such as Sapindaceae, Aceraceae, Hippocastanaceae, Julianaceae and Sabiaceae (Bentham and Hooker 1862; Benson 1970; Hutchinson 1973). Cronquist's (1981) Sapindales include, in addition to the above mentioned families, Burseraceae, Simaroubaceae, Meliaceae and Rutaceae. Almost all these families were grouped by Takhtajan (1980) in his Rutales. The family Anacardiaceae is divided into two tribes namely Anacardieae and Spondieae by Hooker (1872) based on the number of locules in the ovary. The tribe Spondieae was accepted by Engler and Prantl (1895) but they redistributed the taxa belonging to the tribe Anacardieae into 4 smaller tribes (i) Mangifereae (Anacardieae), (ii) Rhoideae, (iii) Semecarpeae and (iv) Dobineae.

The various taxa included in this family show a considerable variation and reduction especially in the number of stamens and carpels. According to Hallier (1905), Anacardiaceae have been evolved from Rutaceae and form the basic stock from which the families of Amentiferae and Aceraceae have been originated. Due to the tendency of the taxa to evolve unisexual flowers and compound leaved members, Hutchinson (1973) recognised Anacardiaceae as one of the advanced families of Sapindales. Within the family, the genus *Buchanania* is the most primitive genus in having pentamerous flowers with apocarpus pistil, 10 fertile stamens and simple alternate thickly coriaceous leaves. Between the monocarpellate members, *Anacardium* and *Mangifera*, the latter, due to the higher degree of sterility, is considered more advanced. Among the syncarpus group, *Semecarpus*, having 5 pistils (indicating the number of carpels) is primitive to *Schinus* and *Choerospondias*. Due to derived tetramerous conditions, *Lannea* is also considered one of the most advanced taxa.

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Earlier chemical reports from the family are: mangiferin from the root bark of *Mangifera*; quercetin, myricetin and apigenin glycosides from the leaves of *Rhus* spp., robustoflavone from seed kernal of *Rhus* (Yuh-Meei and Fa Ching 1974), biflavones of *Rhus* (Fa-Ching and Yue-Meei 1975) and *Semecarpus* (Rao *et al* 1973) and fustin and fisetin from heartwood of *Rhus* (Fa-Ching *et al* 1974).

In the present work, 19 members belonging to Anacardiaceae have been analysed for phenolic compounds such as flavonoids, coumarins and phenolic acids. These plants have also been screened for tannins, saponins, alkaloids and iridoids.

2. Materials and methods

The plants were collected from various localities of India, such as Baroda, Panchamarhi, Kashmir, Kerala and Calcutta. Voucher specimens are deposited in the Herbarium of the University. Mature leaves were selected for the studies and standard procedures (Mabry *et al* 1970; Harborne 1984) were followed for the isolation and identification of various compounds.

3. Results

The distribution of various flavonoids, phenolic acids, saponins and tannins in 19 members of the Anacardiaceae is presented in table 1.

All the 19 taxa screened contained flavonoids in the leaves. Flavonols form the major pigments in the family. The various flavonols encountered are kaempferol, fisetin, quercetin, myricetin and their methoxylated derivatives. Quercetin and its derivatives are located in most of the taxa studied. Myricetin is fairly common and fisetin, kaempferol and its derivatives are less frequent. Flavones such as apigenin and its 7- and 4′-methoxylated derivatives are obtained from *Rhus paniculata*, *Dracontomelum mangiferum* and *Pleiogynium timoriense*. Glycoflavones are located in *Rhus paniculata*, *Semecarpus subpanduriformis*, *Dracontomelum mangiferum* and *Chaerospondias axillaris*. The glycoflavones identified are 4′-OCH_3 vitexin and its isomer 4′-OCH_3 isovitexin. Mangiferin- the C-glyosyl xanthone is present in both the species of *Mangifera*. Except *Pleiogynium* all the taxa contain proanthocyanidins. Tannins are common, saponins rare, alkaloids and iridoids are absent.

4. Discussion

The common constituents of the Anacardiaceae are the highly hydroxylated phenolic compounds such as quercetin, myricetin and gallic acid. The most obvious feature which the members of this family have in common is the presence of proanthocyanidins and flavonols. Though the number of plants screened is not quite large, the

Table 1. The distribution of various plant products in some members of Anacardiaceae.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Tribe Mangiferae																												
Anacardium occidentale Linn									+		+					+	+		+	+			+		+		+	+
Buchananai lanzan Spreng.						+	+		+		+			+		+			+	+	+				+			+
Lannea coromandelica Merr.									+					+		+			+	+		+			+			+
Mangifera caloneura Kurz								+	+						+	+			+	+		+						+
M. indica Linn.						+		+	+		+	+	+		+	+			+	+								
Tribe Rhoideae																												
Rhus mysorensis Heyne									+			+	+			+			+	+		+						+
R. paniculata Wall.	+	+		+				+								+			+	+		+						+
R. parviflora Roxb.									+		+			+		+			+	+	+				+	+		+
R. succedenea Linn.							+		+		+			+		+	+			+			+					+
Schinus molle Hort.									+					+		+			+	+	+		+					+
S. terebinthifolius Raddi							+		+					+		+				+	+		+					+
Tribe Semecarpeae																												
Semecarpus anacardium Linn.						+	+							+		+			+	+	+	+	+					
S. subpanduriformis Wall.				+	+	+		+						+		+		+	+	+			+					+
Holigarna arnottiana Hook.									+				+	+		+			+	+	+		+	+				+
Tribe Spondieae																												
Dracontomelum mangiferum Blume			+		+	+		+			+	+				+			+	+								+
Chaerospondias axillaris (Roxb) Burtt. and Hill.					+			+	+				+	+		+	+		+	+	+							
Pleiogynium timoriense Lee	+																+	+	+	+						+		
Sclerocarya caffara Sond.						+										+				+			+					
Spondias pinnata (Linn.f.) Kurz.									+		+	+	+			+	+	+	+						+			+

1, Apigenin; 2, 7-OCH_3 apigenin; 3, acacetin; 4, 4′-OCH_3 vitexin; 5, 4′-OCH_3 isovitexin; 6, kaempferol; 7, 4′-OCH_3 kaempferol; 8, fisetin; 9, quercetin; 10, 3-OCH_3 quercetin; 11, 3′-OCH_3quercetin; 12, 7-OCH_3 quercetin; 13, 3′, 4′-DiOCH_3 quercetin; 14, myricetin; 15, mangiferin; 16, pronathocyanidins; 17, *p*-hydroxy benzoic acid; 18, protocatechuic; 19, vanillic; 20, syringic; 21, melilotic; 22, gentisic; 23, gallic; 24, *β*-resorcyclic; 25, *p*-coumaric; 26, *o*-coumaric; 27, saponins; 28, tannins.

observations might prove, in the future, to have special taxonomic significance. The chemical differences among the tribes are not very pronounced, however none of the taxa of the tribe Mangiferae contains flavones, either as O-glycosides or C-glycosides. Mangiferin, a C-glycosidic xanthone, also is restricted to the two species of *Mangifera* within the tribe. On the other hand, flavones are more frequent in the tribe Spondieae. Both these tribes have lesser frequency of myricetin and gallic acid and higher incidence of fisetin. The common occurrence of flavonols with a trihydroxy substituted B-ring, namely myricetin, and gallic acid in the tribes Rhoideae and Semecarpeae is noteworthy.

Within the tribe Mangiferae, the genus *Mangifera* appears to be the most advanced member in the absence of both myricetin and gallic acid and in the presence of methoxylated derivatives of quercetin. In containing the former compounds, *Buchanania* can be considered the most primitive taxon.

Spondias axillaris Roxb., which is separated and elevated to a new genus *Chaerospondias* (*C. axillaris* (Roxb.) Burtt. and Hill.), is chemically different from *Spondias pinnata*, with which it was associated earlier. The former taxon contained fisetin, myricetin syringic acid and melilotic acid as against, 4′- and 3′,4′-dimethoxy quercetin, protocatechuic and *p*-coumaric acids of the latter species. These differences corroborate the morphological differences existing between the two taxa and the concept of *Chaerospondias* away from *Spondias* (Mukherjee and Chandra 1983) gains more support.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 3, June 1988, pp. 209–214.
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Influence of rice crop canopy on growth and nitrogen fixation of water fern *Azolla pinnata*

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MS received 31 August 1987

Abstract. Effect of the rice crop canopy was studied as regards the availability of solar radiation to *Azolla* (*Azolla pinnata* R Brown) and its growth and nitrogen fixation while using late inoculation of 0·5 or 1 t fresh fronds/ha during both wet and dry seasons. The higher leaf area index of rice due to increasing rates of fertilizer-N application caused more shading to the *Azolla* plants and reduced their biomass and fixed nitrogen, irrespective of the season. The biomass and nitrogen content of *Azolla* were higher using an inoculum of 1 t/ha than using 0·5 t/ha. *Azolla* growth and fixed nitrogen in treatments of 40 and 80 kg N/ha urea were comparatively higher when rice seedlings were transplanted with double narrow-row spacing than that of normal spacing.

Keywords. *Azolla pinnata*; N_2-fixation; rice crop canopy; shading effects.

1. Introduction

The water fern *Azolla* fixes atmospheric nitrogen through its symbiotic association with a heterocystous blue-green alga, *Anabaena azollae*, which remains in the cavities of the dorsal leaf lobes of the fern (Becking 1979; Lumpkin and Plucknett 1980; Peters *et al* 1982). Its potential as a source of organic nitrogen for rice production is established (Liu 1979; Singh 1979a, b, 1982a, b; Talley and Rains 1980; Watanabe 1982; Singh and Singh 1986). *Azolla* is either multiplied in fallow rice fields and incorporated into the soil before transplanting of rice seedlings or grown as an intercrop with rice. During intercropping, density of rice crop considerably affects growth and N_2-fixation of the fern. Both biomass and fixed N in *Azolla* are less in transplanted than in fallow fields (Rains and Talley 1979), and reduction in biomass is more when inoculation is delayed (Singh and Singh 1987). The present study describes the effect of varying densities of rice canopy on the availability of solar radiation to *Azolla* growing under the rice crop and its growth and N_2-fixation.

2. Materials and methods

A field study was conducted at the Institute farm during wet (June to October) 1984 and dry (January to May) 1985 seasons. The soil of the experimental plots was clay loam with pH 6·5, organic C 0·79%, total N 0·09% and available P 10 mg/kg. Rice (*Oryza sativa* Linn., cv. Jaya of 135 days) seedlings of 30-day-old were transplanted with normal (15 × 20 cm) and double narrow-row (15 × 7–15 × 40 cm) spacings, and 0, 40 or 80 kg N/ha as urea was applied to obtain varying densities of the canopy. Half the nitrogen was applied before transplanting and a quarter each 20 and 60 days after transplanting (DAT). In double narrow-row spacing, the pair of rows were spaced at 40 cm with 7 cm gap between two narrow rows and hills were kept at a distance of 15 cm. *Azolla* was grown in the broader space between two pairs of narrow rows. *A. pinnata* R.Br. (Thailand isolate) was inoculated at 0·5 or 1 t fresh fronds/ha 30 DAT, to produce the maximum effect of rice shading on *Azolla*. The spacing, nitrogen and *Azolla* inoculum treatments were assigned to main, sub and sub-sub plots, respectively, in a split-split plot design with 3 replications. Superphosphate at 4·4 kg P/ha was applied in 3 equal splits 0, 7 and 14 days after inoculation (DAI) to increase the *Azolla* growth. To protect *Azolla* from insect pests, 90 g carbofuran/ha was applied once during inoculation.

Growth (fresh weight t/ha) and N_2-fixation (kg N/ha) of *Azolla* were recorded 60 DAT from 10 random samples in each plot, collected with the help of a 25 × 25 × 25 cm metal frame. Total nitrogen was estimated by the microkjeldahl method (Jackson 1967). Light intensity on top of the rice canopy and above the *Azolla* surface (between 4 rice hills) was measured by a Toshiba Photocell illuminometer (SPI-5) at 8, 12 and 16 h of the day from 5 places 30, 40, 50 and 60 DAT. The percentage reductions in the light intensity reaching above the *Azolla* plants of those above the rice canopy were calculated. Leaf area index (LAI) of rice was recorded from the samples of 5 hills at these stages.

3. Results

3.1 *Shading to Azolla plants*

A significant reduction in light intensity was observed with the increasing doses of urea application at all the stages of crop (30–60 DAT), irrespective of seasons (table 1). Rice crop planted with normal spacing caused 4·4–7·7% greater mean reduction in light intensity during *Azolla* growth than the double narrow-row planting. However, two spacing treatments were statistically at par with respect to shading.

3.2 *Growth and N_2-fixation of Azolla*

During both wet and dry seasons, increase in the rate of fertilizer-N application significantly reduced growth and N_2-fixation of *Azolla* (table 2). *Azolla* biomass and N_2-fixed in 1 t/ha inoculum were higher than in 0·5 t/ha inoculum treatment. Rice fields planted with double narrow-row spacing recorded the increase of 14·8–17%

Table 1. Effect of nitrogen levels on per cent shading to *Azolla* (mean of spacing and inoculum treatments).

	Wet season				Dry season			
	Days after transplanting				Days after transplanting			
Treatments	30	40	50	60	30	40	50	60
No nitrogen	45·0	49·6	53·1	54·4	41·1	45·6	48·8	50·0
40 kg N/ha	50·8	57·4	59·9	63·1	46·9	53·4	55·8	58·7
80 kg N/ha	55·0	64·2	65·3	67·6	52·5	62·6	62·2	65·2
CD 5%	1·0	1·4	2·2	1·5	1·2	1·2	1·5	1·1

Table 2. Effect of spacings, nitrogen levels and *Azolla* inocula on the growth and N_2-fixation of *Azolla*.

	Wet season				Dry season			
	Azolla fresh wt. (t/ha)		N_2-fixed (kg N/ha)		*Azolla* fresh wt. (t/ha)		N_2-fixed (kg N/ha)	
Treatments	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
No nitrogen								
0·5 t *Azolla*/ha	7·65	7·44	16·37	16·03	8·24	8·20	17·63	16·68
1·0 t *Azolla*/ha	7·92	7·70	17·01	16·55	8·45	8·48	18·16	18·21
40 kg N/ha								
0·5 t *Azolla*/ha	5·44	6·51	11·54	13·84	6·02	7·15	12·75	15·72
1·0 t *Azolla*/ha	6·16	6·80	13·05	14·45	6·53	7·55	13·85	16·07
80 kg N/ha								
0·5 t *Azolla*/ha	4·52	4·90	9·47	10·25	5·10	5·53	10·78	11·55
1·0 t *Azolla*/ha	5·14	5·60	10·78	11·73	5·52	6·18	11·62	12·92
CD 5%*								
Spacing	NS		NS		NS		NS	
Nitrogen	0·27		0·61		0·30		0·64	
Nitrogen at same spacing	0·39		0·86		0·43		0·91	
Spacing at same or different nitrogen	3·82		7·90		4·30		9·07	
Azolla	0·21		0·48		0·26		0·55	

*Other interactions nonsignificant.

S_1 normal spacing (15 × 20 cm), S_2 double narrow-row spacing (15 × 7 cm – 15 × 40 cm).

NS Not significant.

Table 3. Effect of nitrogen levels on leaf area index of rice (mean of spacing and inoculum treatments).

	Wet season				Dry season			
	Days after transplanting				Days after transplanting			
Treatments	30	40	50	60	30	40	50	60
No nitrogen	0·88	1·17	1·63	1·90	0·64	0·88	1·24	1·44
40 kg N/ha	1·07	1·46	2·11	2·48	0·79	1·11	1·61	1·91
80 kg N/ha	1·32	1·81	2·44	2·91	1·07	1·46	1·97	2·34
CD 5%	0·07	0·10	0·12	0·15	0·10	0·12	0·20	0·25

and 8·7–10·3% in *Azolla* biomass and 15–17·7% and 8·5–9·3% in total N_2-fixed over the normal spacing in treatments of 40 and 80 kg N/ha respectively. However, the differences between two spacing treatments were nonsignificant.

3.3 *LAI*

The LAI of the rice crop at all stages during *Azolla* growth (30–60 DAT) increased significantly with increase in the dose of fertilizer-N application (table 3) during both wet and dry seasons. In treatments of zero N at all stages and 40 kg N/ha up to 40 DAT, LAI was lower when rice was planted in double narrow-rows as compared to the normal spacing. However, spacing treatments did not differ significantly.

4. Discussion

Urea was applied at the rate of 0, 40 or 80 kg N/ha to produce varying levels of rice canopy. *Azolla* growth was shown to be affected a little by the split application of ammonium sulphate or urea up to 90 kg N/ha (Singh 1982b; Satapathy and Singh 1985). Further, Singh *et al* (1981) while using ammonium sulphate reported the release of about 87% of NH_4^+-N on 10th day of flooding which gradually decreased and reached to a minimum of 6% on 50th day. In the present experiment, half nitrogen was applied before transplanting and a quarter each at 20 and 60 DAT, and *Azolla* was applied at 30 DAT. Thus, the possible effect of fertilizer-N on the growth of *Azolla* was minimized.

The delay in *Azolla* application in rice fields caused significant reduction in *Azolla* biomass, its N content and reductions were more when *Azolla* was applied 4 weeks after transplanting (WAT) while yield of rice increased with *Azolla* application up to 3–4 WAT (Singh and Singh 1987). In this study, *Azolla* was inoculated with 30 DAT to produce maximum effect of rice shading. The increase in LAI of rice observed in this trial during *Azolla* growth with the increasing rates of fertilizer-N application caused more shading to the *Azolla* plants and possibly resulted in its poor growth and N_2-fixation. The per cent reduction in light intensity available above the *Azolla* surface of that failing on top of rice canopy was negatively correlated with both biomass ($r = -0{\cdot}87^{**}$ in wet and $-0{\cdot}92^{**}$ in dry season) and N yields ($r = -0{\cdot}89^{**}$ in wet and $-0{\cdot}91^{**}$ in dry season) of *Azolla.* Lu *et al* (1963) studied the effect of varying light intensities on *A. pinnata* and observed maximum biomass with higher rate of N_2-fixation at 47 K lux. The growth rate and nitrogenase activity of *A. filiculoides* were maximum at 50% full sun light (about 49 K lux) (Ashton 1974). However, Peters (1976) reported from laboratory studies that light saturation of *A. caroliniana* occurred at much lower intensities (4·8 K lux). The biomass of *A. filiculoides* and *A. mexicana* were reported to be higher in open fields than in dual cropping even with a light rice cover (Rains and Talley 1979).

In this trial, maximum light intensities of 82–108 and 89–112 K lux were recorded during *Azolla* growth on the top of rice canopy at noon in wet and dry seasons respectively, while intensities during morning and evening hours were much lower. Mean reduction in light intensity during *Azolla* growth was 50·6, 57·9, 63·1% and 46·5, 53·9, 60·8% in treatments of 0, 40 and 80 kg N/ha during wet and dry seasons respectively. Photosynthesis and related physiological processes of *Azolla* might become saturated at relatively lower light intensities. However, in a well developed *Azolla* mat crowding may become intense as to cause significant shading of individual fronds and keep the photosynthetic capacity of the whole mat well below the optimum even in full sun light. This could possibly explain the effect of rice canopy on biomass production of *Azolla.*

Azolla biomass and N yields in 1 t/ha inoculum were more than in the inoculum of 0·5 t/ha. Higher inoculum density is better for *Azolla* growth in transplanted (Singh 1984) as well as in fallow fields (Dao and Tran 1979). The increments of 14·8–17% and 8·7–10·3% in *Azolla* biomass and 15–17·7% and 8·5–9·3% in total N_2-fixed were obtained in treatments of 40 and 80 kg N/ha respectively, when rice was planted in double narrow-row as compared to the normal planting. This could be due to relatively lower reduction in light intensity and availability of more space in the former spacing treatment. However, the differences between two spacings were nonsignificant possibly because of less precision by assigning the spacing treatments in the main plots of a split-split plot design. Singh *et al* (1982) reported that *Azolla* growth was little affected by different spacings when inoculated just after planting, but double narrow-row spacing produced more *Azolla* biomass in inoculations at later stages.

It was concluded from this study that higher LAI of rice due to increasing rates of fertilizer-N application caused more shading to *Azolla* plants and reduced their growth and N_2-fixation.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 3, June 1988, pp. 215–226.
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Pattern of immobilization and release of nitrogen in decomposing leaf litter in Himalayan forests

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MS received 30 April 1987; revised 20 January 1988

Abstract. The nitrogen dynamics in decomposing leaf litter, incubated on different forest sites were observed for a two year period. There existed an inverse relationship between the per cent dry matter remaining and its nitrogen concentration ($r = -0{\cdot}713$ to $-0{\cdot}966$). Litter, placed at sal, pine-mixed broadleaf and mixed oak forest sites showed larger slopes (showing greater weight loss per unit of nitrogen immobilization) compared to pine and mixed oak-pine forest sites. The magnitude of immobilization of nitrogen was different among species at different forest sites. The decomposition constant (k) for organic matter ranged from $-1{\cdot}29$ to $-4{\cdot}61$ and for N release $-1{\cdot}05$ to $3{\cdot}91$. Analysis of variance indicated significant differences in N concentration due to days, species and species × days interaction ($P < 0{\cdot}01$).

Keywords. Decomposition; immobilization; Himalayan forests; leaf litter; nitrogen concentration; decomposition constant.

1. Introduction

Decomposition processes play an important role in soil fertility, in terms of nutrient cycling and maintenance of organic matter level. The mineralization of elements through the decay of organic matter represents a large, available source of nutrients for plants (Clarke 1977; Jorgensen *et al* 1980). Slow decay rate can produce nutrient limitations to primary producers by accumulating large nutrient stock in soil surface. Nitrogen and lignin are the major substrate quality components controlling the rate of decomposition (Staaf and Berg 1982; Berg and Wessen 1984; Mellilo *et al* 1982). Plant nutrients are released from litter either by physical leaching or by breakdown of structural organic components by saprotrophic organisms. Often the later process is quantitatively more important, and the rate at which elements are released could thus be expected to be governed largely by the decomposition rate. The present study describes (i) the changes in the concentration and pattern of release of nitrogen and (ii) influence of initial substrate quality on immobilization and release of decomposing litter from a number of species from 5 forest ecosystems of Kumaun Himalaya.

2. Study sites

2.1 *Location*

The experimental sites are located in the north western part of the central Himalaya (29° 7′ to 29° 26′ N lat. and 79° 15′ to 79° 38′ E long.) and range from 330–2150 m in altitude.

2.2 *Climate*

Based on the climatic variations in central Himalaya, the year is divisible into 3 seasons: rainy (July–October), winter (November–February) and summer (March–June).

The sal forest site is situated at 330 m elevation and represents the warmest habitat whereas mixed oak forest site (2150 m) represents the coldest. Mean daily temperature ranges between 12–32°C (sal) and 4·5–22°C (mixed oak). The temperature of remaining 3 sites fall between these two. Mean annual rainfall is 2076, 2005, 2185, 1313 and 2488 mm respectively, at Sal, pine-mixed broadleaf, pine, mixed oak-pine and mixed oak forest sites, 76–88% of which occurs in the rainy season.

2.3 *Geology*

Sal and pine-mixed broadleaf forest sites are situated on Siwalik rocks. The rocks mainly comprise thickly-bedded hard, compact sandstone associated with purple, red and green slates. The pine forest site, being situated in the northern proximity of main boundary thrust, has purple, and green slates interbedded with yellowish weathering dolomite (middle Krol) or greyish green calcareous slates with a thin layer of marl (lower Krol). At the mixed oak-pine forest site the exposed rocks are grey to greyish green garnetiferous mica schist and granitic gneisses. The rocks exposed at the mixed oak forest site are of Blaini formation and comprise pink dolomitic limestone with rounded to well-rounded clasts of quartzite, purple slate and silt stone (A K Sharma, unpublished results).

2.4 *Site characterization*

Soil on the sal forest site is deep and alluvial, while that at higher altitudes is shallow and residual. The coarse particles in soil in 0–30 cm soil cores were negligible at sal forest sites compared to remaining sites (11–28%). Soil texture was determined by soil hydrometer (Buoyoucos 1951), organic carbon by Walkley and Black's (1934) rapid titration method and total nitrogen by Kjeldahl method as described by Piper (1944). Sand predominates in the soil (61–82%) on all sites, while the silt and clay percentages range between 12–26 and 6–16 respectively. Across the sites, organic carbon ranged between 1·10–4·20% and total nitrogen between 0·15–0·40% (table 1).

3. **Materials and methods**

3.1 *Leaf litter decomposition*

Mature nearly senesced but attached leaves were collected from the middle canopy of trees of selected species in June–July 1981 and air-dried in shade. Leaf fall in the present forests occurs throughout the year although the peak fall occurs during the summer season (Pandey and Singh 1982; Mehra 1984).

The litter bag technique was used for quantifying the rate of decomposition. Nylon netting litter bags (1 mm mesh) of 10 × 10 cm size, containing 5 g air-dried leaf litter were placed on the forest floor of the forest sites (table 2). Although, various methods

Table 1. Some physical and chemical properties of soils in the study sites.

	Forest				
	Sal	Pine-mixed broadleaf	Pine	Mixed oak-pine	Mixed oak
Colour	Dark brown	Dark brown	Dark brown	Dark yellowish brown	Dark brown
Texture (%)					
Sand	80	82	66	61	62
Silt	14	12	24	26	22
Clay	6	8	10	13	16
Organic carbon (%)	1·08	3·54		3·54	4·20
Total nitrogen (%)	0·15	0·30	0·26	0·33	0·46
C/N ratio	7·13	11·60	14·27	10·40	9·21

Table 2. Date of litter bag placing and number of litter bag placed for species at different forest sites.

Forest type	Leaf litter species	Numbers of bags	Date placed
Sal	*Shorea robusta*	120	7 July 1981
	Mallotus philippensis	80	
	Quercus leucotrichophora	120	
Pine-mixed broadleaf	*Lyonia ovalifolia*	120	7 July 1981
	Quercus glauca	80	
	Rhododendron arboreum	80	
	Quercus leucotrichophora	120	
Pine	*Pinus roxburghii*	120	7 August 1981
	Quercus leucotrichophora	120	
Mixed oak-pine	*Quercus leucotrichophora*	120	7 August 1981
	Myrica esculenta	80	
Mixed oak	*Quercus lanuginosa*	120	8 July 1981
	Quercus floribunda	80	
	Quercus leucotrichophora	120	

available for investigating decomposition rates have several drawbacks. The use of litter bags provides a standardised basis for comparisons among species, sites and experimental manipulations (Singh and Gupta 1977; Wieder and Lang 1982).

Litter bags of *Quercus leucotrichophora* were placed on the forest floor of each site. The leaf litter of *Q. leucotrichophora*, collected from its native site, was used as a standard material for investigating the effect of habitat on decomposition.

On the sal forest site, steel wire netting bags with the same mesh size and painted with synthetic enamel to avoid rusting, were used because termites damage nylon bags. Five replicates, 5 g air-dried weight each, from stock litter samples were dried at 70°C to determine a correction factor for estimating the initial oven dry weight of litter in bags.

Five litter bags were randomly recovered (for those species where 120 bags placed) at one month interval over a year. Two litter bags were used to determine the microbial and microfaunal populations of the decomposing litter in a related study (Pande 1984; Sharma *et al* 1984). For the remaining species only 3 bags were recovered at one-month intervals over a year. In a number of species the decomposition was not completed over a year. In these cases the bags were recovered beyond one year until the litter had disappeared or in one species (*Pinus roxburghii*), till the second year was complete. The litter bags immediately after recovery were placed in individual polyethylene bags and transported to the laboratory. In the laboratory the residual litter was separated from the bags and was carefully cleaned to remove the attached soil particles and weighed. This material was then oven-dried at 70°C to constant weight to calculate the weight loss and moisture content.

3.2 *Chemical analysis*

Total (Kjeldahl) nitrogen (Piper 1944) was determined in litter samples collected at 2-month intervals over the first year. Two further N analysis were carried out after 16 and 19½ months for species with significant residual masses of litter material. By combining these results with the data on weight loss the N mobilisation after different incubation periods were calculated. Annual decomposition coefficients (k) for nitrogen were calculated [$Xt/Xo = e^{-kt}$; where Xt = mass at time t, and Xo = initial mass and k = the annual exponential (base e) decay coefficient] following Olson (1963) after the last chemical analysis for each species and for organic matter after 100% decomposition was achieved (i.e. 99% of the initial weight had disappeared).

4. **Results**

4.1 *Changes in N concentration*

4.1a *Sal forest*: There was a continuous increase in N concentration of the residual litter throughout the decomposition period in all species, although the magnitude of the increase differed among species (table 3). The increase was greatest in *M. philippensis* and was followed by *S. robusta* and *Q. leucotrichophora*. At the end of the first year N concentration was 2·34 times that of the initial value for *S. robusta*, 3·42 times for *M. philippensis* and 1·82 times for *Q. leucotrichophora*.

4.1b *Pine-mixed broadleaf forest*: In the initial period of decomposition the increase occurred in *Q. glauca*, as after 62 days, N concentration reached 1·87% from the initial 0·94%. For the same period N concentration was 1·05% (initial 0·80%) for *L. ovalifolia*, 1·12% (initial 0·70%) for *R. arboreum* and 1·23% (initial 1·15%) for *Q. leucotrichophora*. After 487 days the N increase in *R. arboreum* was 2·40 times and in *Q. leucotrichophora* 2·10 times that of the initial concentration. Between 487 and 578 days, there was no increase in N concentration in the residual material of *R. arboreum*. Between these periods the decomposition of *Q. leucotrichophora* was complete.

4.1c *Pine forests*: The increase in N concentration was slightly greater in *P. roxburghii* (1·94 times) compared to *Q. leucotrichophora* (1·45 times of the initial) in the first year. The increase in N concentration at the end of 578 days was

Table 3. Changes in dry weight and N content of the leaf litter from several tree species, placed on different forest sites for different periods of decomposition.

Period after placement of bags (days) (1)	Weight of litter remaining (%) (2)	Weight of N			Observed weight of N as per cent of initial (6)	Change in N content (%) (7)
		(g) (3)	(%) (4)	(mg) (5)		
			Sal forest			
S. robusta						
Initial	100	4·58	0·99	45	—	—
62	73·76	3·38	1·36	46	102	2
123	54·56	2·50	1·40	35	78	−22
184	47·21	2·16	1·45	31	69	−31
243	36·76	1·68	2·00	34	76	−24
304	15·42	0·71	2·22	16	36	−64
365	3·68	0·17	2·32	4	8	−92
	Total decomposition occurred in 396 days					
M. philippensis						
Initial	100	4·68	0·52	24	—	0
62	56·05	2·62	0·85	22	92	−8
123	38·42	1·80	1·20	22	92	−8
184	20·67	0·91	1·22	12	50	−50
143	10·51	0·49	1·64	8	33	−67
304	3·77	0·18	1·78	3	13	−87
	Total decomposition occurred in 365 days					
Q. leucotrichophora						
Initial	100	4·55	1·15	52	—	0
62	55·19	2·51	1·23	31	60	−40
123	38·49	1·75	1·40	25	48	−52
184	29·80	1·36	1·49	20	38	−62
243	16·38	0·75	1·62	12	23	−77
304	5·47	0·25	1·75	4	8	−92
365	1·26	0·06	2·09	1	2	−98
	Total decomposition occurred in 396 days					
L. ovalifolia						
Initial	100	4·68	0·80	37	—	0
62	45·52	2·13	1·05	22	59	−41
123	38·33	1·79	1·13	20	54	−46
184	31·10	1·46	1·17	17	46	−54
243	19·42	0·91	1·25	11	30	−70
304	14·02	0·66	1·72	11	30	−70
365	3·71	0·17	1·89	3	8	−92
	Total decomposition occurred in 396 days					
Q. glauca						
Initial	100	4·65	0·94	44	—	—
62	52·92	2·46	1·87	46	105	5
123	30·62	1·42	1·94	28	64	−36
184	24·41	1·14	2·12	24	55	−45
243	20·14	0·94	2·14	20	45	−55
304	10·91	0·51	2·24	11	25	−75
	Total decomposition occurred in 365 days					
R. arboreum						
Initial	100	4·85	0·70	34	—	–
62	75·74	3·67	1·12	41	121	
123	63·57	3·08	1·20	37	109	

Table 3. (*Contd.*)

(1)	(2)	(3)	(4)	(5)	(6)	(7)
184	59·86	2·90	1·24	36	106	6
243	55·22	2·68	1·26	34	100	0
304	40·09	1·94	1·30	25	74	−26
365	27·59	1·34	1·52	20	59	−41
487	9·74	0·47	1·68	8	24	76
578	4·54	0·22	1·68	4	12	−88
Total decomposition occurred in 608 days						
Q. leucotrichophora						
Initial	100	4·55	1·15	52	—	0
62	68·92	3·14	1·23	39	75	−25
123	60·20	2·74	1·34	37	71	−29
184	51·30	2·33	1·53	36	69	−31
243	44·26	2·01	1·74	35	67	−33
304	27·74	1·26	1·78	22	42	−58
365	12·95	0·59	1·95	12	23	−77
487	2·55	0·12	2·42	3	6	−94
Total decomposition occurred in 520 days						
Pine forest						
P. roxburghii						
Initial	100	4·52	0·67	30	—	0
62	80·48	3·64	0·79	29	97	−3
123	79·00	3·57	0·97	35	117	17
184	73·31	3·31	1·01	33	110	10
243	64·96	2·94	1·15	34	113	13
304	55·88	2·53	1·24	31	103	3
365	48·73	2·20	1·30	29	97	−3
487	33·71	1·52	1·35	21	70	−30
578	28·23	1·28	1·39	18	60	−40
730	7·56	0·34	1·39	5	17	−83
Q. leucotrichophora						
Initial	100	4·55	1·15	52	—	0
62	75·52	3·44	1·14	39	75	−25
123	73·58	3·35	1·20	40	77	−23
184	71·96	3·27	1·26	41	79	−21
243	51·53	2·34	1·35	32	62	−38
304	41·26	1·88	1·42	27	52	−48
365	26·07	1·19	1·67	20	38	−62
487	13·36	0·61	1·82	11	21	−79
578	9·22	0·42	2·12	9	17	−83
Total decomposition occurred in 669 days						
Mixed oak-pine forest						
Q. leucotrichophora						
Initial	100	4·55	1·15	52	—	0
62	70·13	3·19	1·16	37	71	−29
123	64·40	2·93	1·16	34	65	−35
184	56·64	2·58	1·20	31	60	−40
243	45·85	2·09	1·31	27	52	−48
304	37·22	1·69	1·84	31	60	−40
365	23·62	1·07	1·97	21	40	−60
487	8·56	0·39	2·12	0	15	−85
578	3·27	0·15	2·27	3	6	−94
Total decomposition occurred in 609 days						

Table 3. (*Contd.*)

(1)	(2)	(3)	(4)	(5)	(6)	(7)
M. esculenta						
Initial	100	4·72	0·58	27	—	0
62	87·70	4·14	0·68	28	104	4
123	87·68	4·14	0·81	34	126	26
184	66·69	3·15	0·85	28	104	4
243	61·97	2·92	0·97	28	104	4
304	43·24	2·04	1·05	21	78	−22
365	26·84	1·27	1·12	14	52	−48
487	14·08	0·66	1·20	8	30	−70
578	7·96	0·38	1·25	5	19	−81
	Total decomposition occurred in 639 days					
	Mixed oak forest					
Q. lanuginosa						
Initial	100	4·64	1·32	61	—	0
62	75·78	3·52	1·76	62	102	2
123	67·31	3·12	1·45	45	74	−26
184	62·52	2·90	1·41	41	67	−32
243	51·12	2·37	1·44	34	56	−44
304	41·42	1·92	1·84	35	57	−43
365	32·07	1·49	2·66	40	66	−34
487	12·09	0·56	2·87	16	26	−74
578	6·47	0·30	3·12	9	15	−85
	Total decomposition occurred in 669 days					
Quercus floribunda						
Initial	100	4·67	0·97	45	—	0
62	76·35	3·57	1·12	40	89	−11
123	70·85	3·1	1·02	34	76	−24
184	65·33	3·05	1·32	40	89	−11
243	50·77	2·37	1·87	44	98	−2
304	43·23	2·02	2·71	55	122	22
365	35·92	1·68	3·05	51	113	13
487	13·12	0·61	3·10	19	42	−58
578	5·48	0·26	3·15	8	18	−82
	Total decomposition occurred in 669 days					
Q. leucotrichophora						
Initial	100	4·55	1·15	52	—	0
62	63·68	2·90	1·18	34	65	−35
123	51·82	2·36	1·32	31	60	−40
184	50·00	2·28	1·12	26	50	−50
243	41·36	1·88	1·33	25	48	−52
304	24·86	1·13	1·68	19	37	−63
365	12·87	0·59	2·55	15	29	−71
487	1·27	0·06	2·68	2	4	−96
	Total decomposition occurred in 548 days					

P. roxburghii (2·09 times greater than the initial) and *Q. leucotrichophora* (1·84 times from the initial) (table 3).

4.1d *Mixed oak-pine forest*: Increase in N concentration was greater in *M. esculenta* than in *Q. leucotrichophora*. After 365 days the increase was 1·71 and

1·93 times and after 578 days it was 1·97 and 2·16 times of the initial value for *Q. leucotrichophora* and *M. esculenta* respectively (table 3).

4.1e *Mixed oak forest*: The litter of oak species showed an increase in N concentration in initial periods of decomposition followed by a decrease in the winter season. After the first winter all species exhibited an increase in N concentration (table 3). The increase was more than 3 times in *Q. floribunda* at the end of the first year. For the same period increase was 2·02 and 2·22 times of the initial in *Q. lanuginosa* and *Q. leucotrichophora* respectively. In *Q. leucotrichophora* N concentration reached 2·68% (initial 1·15%) in 487 days of litter incubation. After 578 days, N concentration was 3·12% compared to the initial 1·32% for *Q. lanuginosa* and 3·15% compared to the initial 0·97% for *Q. floribunda*.

Thus, varying degrees of N immobilization were observed for the species at different forest sites and the magnitude of immobilization was different among species. Analysis of variance indicated significant differences in N concentration due to days and species ($P<0{\cdot}01$) across sites. A significant interaction ($P<0{\cdot}01$) between species and days indicates that the behaviour of litter species in this regard varied across dates.

4.2 *Nitrogen release during decomposition*

4.2a *Sal forest*: Initial release was much faster in *Q. leucotrichophora* compared to *M. philippensis*. *S. robusta* did not release N in initial periods and an absolute increase of 0·21 mg N mass per litter bag occurred after 62 days of litter incubation. In the later periods of the cycle, *S. robusta* showed faster release compared to *M. philippensis* and *Q. leucotrichophora*. However, in the total period faster N release occurred in *Q. leucotrichophora* ($k=3{\cdot}91$) and *S. robusta* ($k=2{\cdot}52$) followed by *M. philippensis* ($k=2{\cdot}49$) (table 4).

4.2b *Pine-mixed broadleaf forest*: N release in initial periods was faster in *L. ovalifolia* and *Q. leucotrichophora*, whereas an increase in absolute mass of N was observed in *Q. glauca* and *R. arboreum*. About 39% of total N was released from *Q. glauca* at the end of 123 days, whereas for the same period in other 3 species the release of N was 4–12% only. However, for the total decomposition cycle N release was faster in *L. ovalifolia* ($k=2{\cdot}53$) followed by *Q. leucotrichophora* ($k=2{\cdot}12$), *Q. glauca* ($k=1{\cdot}69$) and *R. arboreum* ($k=1{\cdot}34$) (table 4).

4·2c *Pine forest*: Initial release was only 3% followed by an increase of 1·32 mg N mass per litter bag, in *P. roxburghii*, whereas initial release was 25% and a net increase of 0·22 mg N mass per litter bag in *Q. leucotrichophora* after 123 days (table 3). In the total decomposition cycle N release was faster in *Q. leucotrichophora* compared to *P. roxburghii* as annual decomposition constants were 1·12 and 0·89 respectively, for the two species (table 4).

4·2d *Mixed oak-pine forest*: *Q. leucotrichophora* exhibited 29% release of total N after 62 days of litter incubation, whereas *M. esculenta* showed 4 and 26% increase in absolute mass after 62 and 123 days respectively (table 3). However, a net increase of 0·88 mg was observed in the release phase of *Q. leucotrichophora* after 304 days. The

Table 4. Annual decomposition constant (k) for nitrogen and organic matter for decomposing leaves of several species from different forest sites.

		k	
Forest site	Species	Organic matter	N
Sal	*S. robusta*	4·25	2·52
	M. philippensis	4·61	2·49
	W. leucotrichophora	4·25	3·91
Pine-mixed broadleaf	*L. ovalifolia*	4·25	2·53
	Q. glauca	4·61	1·69
	R. arboreum	2·77	1·34
	Q. leucotrichophora	3·23	2·12
Pine	*P. roxburghii*	1·29	0·89
	Q. leucotrichophora	2·52	1·12
Mixed oak-pine	*Q. leucotrichophora*	2·76	1·78
	M. esculenta	2·63	1·05
Mixed oak	*Q. lanuginosa*	2·52	1·20
	Q. floribunda	2·52	1·08
	Q. leucotrichophora	3·07	2·42

annual decomposition constant was 1·78 for *Q. leucotrichophora* and 1·05 for *M. esculenta* (table 4).

4.2e *Mixed oak forest*: Decrease in N weight was observed in *Q. floribunda* and *Q. leucotrichophora* in initial periods, whereas *Q. lanuginosa* showed an increase in total weight of N of 0·21 mg. A net increase of 1·29, 0·85 and 2·36 mg N per litter bag was observed in *Q. floribunda* after 184, 243 and 304 days of litter incubation, whereas the other two species showed a continuous release. The annual decomposition constant was 1·20 for *Q. lanuginosa*, 1·08 for *Q. floribunda* and 2·42 for *Q. leucotrichophora* (table 4).

5. Discussion

The increase in N concentration in decomposing litter shown in this study is a generally occurring phenomenon brought about by microbial immobilization of N while C is mineralized (Bocock 1963; Gosz *et al* 1973; Berg and Staaf 1981; Anderson *et al* 1983; Fahey 1983; Vogt *et al* 1983). A decline in N concentration in winter was observed at the mixed oak forest site. This site experiences frequent snowfall in winter and it is probable that the decline represents a flushing by snow melt which increases the biological activity and decay rate. Bogatyrev *et al* (1983) noted that freezing and thawing slightly increased the leaching rate for birch litter. McBrayer and Cromack (1980) also reported a decrease in N concentration in winter periods under snow cover in decomposing oak leaf litter.

Significant negative relationships were found between N concentration in residual material and the per cent weight remaining for all species. The regression parameters for these relationships are given in table 5. Aber and Melillo (1980) and Melillo *et al*

(1982) have emphasized the significance of these relationship in the framework of immobilization and mineralization of N during decomposition. The weight loss in enclosed litter and increased N concentration in the residual material reflect carbon metabolization and N immobilization respectively, by micro-organisms. Aber and Melillo (1980) have generalized that this relationship is inversely linear in case where (i) physical removal of material from bags is minimized, (ii) nitrogen is sufficiently low in concentration in the litter material to be limiting to microbes and (iii) a continuous external source of N is available.

Further, Aber and Melillo (1980) stated that on these sites, where litter feeding activities are high, the physical removal by the animals results in increased variability in the measured N concentrations. They found poorer correlation and increase in the slope on such sites. This could result from the selective feeding by the macrofauna on more palatable high nitrogen material being constantly produced by the microbial growth and activity. Litter, placed on sal, pine-mixed broadleaf and mixed oak forest sites, in the present study exhibited larger slopes compared to pine and mixed oak-pine forest sites. This has come due to greater weight loss of litter in the former species per unit of N immobilization.

On these 3 forest sites micro-arthropod population on the forest floor was greater than the pine and mixed oak-pine forest sites (Sharma *et al* 1984). Invasion of microbial population on the litter bags on these forest sites was also greater compared to species of other forest sites (Pande 1984). Also on these 3 forest sites average annual rainfall was also high, 80–88% of which occurs in the rainy season.

According to Waksman and Gerretsen (1931), for every unit of carbon oxidised by micro-organisms a certain amount of N must be assimilated whether it is present in the form of proteins or protein derivatives of inorganic salts. The sources of N taken up by the decomposing litter are not well established. Bocock (1963) considered atmospheric precipitation, insect frass and plant material falling from the canopy as N sources. Staaf and Berg (1977), however, reported that the amount of N in

Table 5. Regression parameters relating per cent weight remaining (Y) and nitrogen concentration (X %) in residual material.

Forest site	Species	Intercept	Slope	%	't' value	SE of slope	d.f
Sal	*S. robusta*	153·01	−63·01	0·916	7·37	8·54	5
	M. philippensis	125·27	−72·43	0·914	6·56	11·04	4
	Q. leucotrichophora	178·13	−93·23	0·770	4·09	22·77	5
Pine-mixed broadleaf	*L. ovalifolia*	125·68	−69·66	0·713	3·53	19·76	5
	Q. glauca	165·40	−66·97	0·959	9·70	6·89	4
	R. arboreum	178·16	−99·75	0·958	12·55	7·92	7
	Q. leucotrichophora	157·83	−67·71	0·898	7·85	8·62	6
Pine	*P. roxburghii*	159·67	−88·43	0·923	9·14	9·66	7
	Q. leucotrichophora	175·63	−85·16	0·861	6·61	12·89	7
Mixed oak-pine	*Q. leucotrichophora*	138·79	−59·24	0·822	5·69	10·41	7
	M. esculenta	187·48	−140·06	0·966	14·03	9·97	7
Mixed oak	*Q. lanuginosa*	124·10	−37·21	0·769	4·83	7·70	7
	Q. floribunda	110·29	−28·82	0·858	6·51	4·42	7
	Q. leucotrichophora	103·16	−36·81	0·593	2·97	12·72	6

All equations are significant at $P < 0{\cdot}01$.

precipitation could not account for the N accumulated in the Scots-pine needle litter. They suggested that N was taken up by the decomposing litter from older litter on the floor with a higher N content. Increased N mass in the initial periods in most of the species suggests that N was strongly immobilized in microbial tissues (Gosz *et al* 1973; Jorgensen *et al* 1980). Ausmus *et al* (1976) have also found absolute increase and stated that it occurred probably due to progressive reduction in the amount of organic carbon present.

Initial nutrient compositions especially lignin and N exert greater influence on N accumulation (Staaf and Berg 1982; Berg and Wessen 1984). In the present experimental species N accumulation took place for longer periods in *P. roxburghii* and *M. esculenta*. These two species have lowest initial N concentration and highest lignin content compared to other species (Upadhyay and Singh 1985). The lignin fraction have relatively low availability to plants and causes low turnover for these species. All other species except these two have $<17\%$ lignin. Upadhyay and Singh (1985) have also shown that in species having $<17\%$ lignin little or no accumulation of N occurred. The pattern of nitrogen release in *Q. leucotrichophora* at all sites and of *L. ovalifolia* followed the total weight loss pattern during the initial periods of decomposition, suggesting leaching of the mobile N pools i.e. labile fractions of litter. In *M. philippensis* also the release of N was only slightly lower compared to the loss of organic matter. Staaf and Berg (1982) argued that some decomposition of lipophilic substances in the cuticle and membranes may take place even before the litter is effectively wetted. The release phase was dominant in *Q. leucotrichophora*, *L. ovalifolia* and *M. philippensis*, but a temporary net import of N was observed in *Q. leucotrichophora* (at pine and mixed oak-pine forest sites). Similar observations in decomposing pine needle litter were reported by Berg and Staaf (1981). The beginning of a decrease in weight of N after an accumulation suggests the occurrence of mineralization.

Acknowledgement

The author gratefully acknowledges financial support from the Department of Science and Technology, New Delhi.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 4, August 1988, pp. 227–249.
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Computer-assisted image analysis in biological sciences

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MS received 3 June 1988

Abstract. Automated computer-controlled image analysis of digitized video images has developed into a versatile tool useful for many applications. Both on the macroscopic and microscopic scales counting, identification and tracking are facilitated by these techniques which allow a quantitative, fast and objective analysis. Though image analysis has not yet reached the capabilities of the human eye and brain, it helps in both routine analysis and research tasks. Future enhancements made possible by the availability of larger memories and higher processing speeds will enhance the realm of applications. Especially the use of pipeline processing and dedicated hardware for the routines of image manipulation, including averaging and Laplace filtering, will open new fields of use. The most difficult task to master will be the extraction of complex parameters necessary for object recognition in an image, which requires a high degree of built-in intelligence both on the hardware and software level. Another ambitious task to tackle is the analysis of image sequences in order to track several individuals in a population in parallel.

Keywords. Digitization; microscopy; image analysis; video tracking.

1. Introduction

The availability of video techniques and the growing use of inexpensive microcomputers allow to automate the analysis of static or dynamic images for multiple purposes (Shipton 1979). Vision by man and machine differ in many respects and certainly the human visual apparatus and brain are still far superior to machine vision (Poggio 1984). Both the spatial and gray or color shade resolutions of the human eye surpass those of high resolution video cameras and the image processing of the human brain far exceeds the speed and complexity of even the fastest computers (Blake 1987).

However, using image analysis in specific research projects facilitates quantitative analysis and objective interpretation of selected parameters. Digitization of analogue images lends itself to image enhancement (Hawkes 1980; Serra 1980; Kokubo and Hardy 1982) and to the extraction of specific features of interest such as dimensions, areas of a distinct colour or gray shade (McMillan *et al* 1987; Russ and Russ 1987) or movement of individual elements in a sequence of images (Lewandowska *et al* 1979; Turano *et al* 1985; Thurston *et al* 1986).

2. Hardware

Basically any image analysis starts with the digitization of a video image. The video source can be a video tube camera, a charged coupled device (CCD) camera or a recorded video image or sequence of images. The camera may be equipped with a macro objective to record small objects or even be mounted on a microscope to study microscopical objects. The source needs to be selected for the specific requirements such as available irradiation (Hayakawa *et al* 1986). The spatial resolution of the image should meet or exceed the resolution of the digitizer; most commercial cameras have a resolution of 625 lines (PAL, SECAM) or 525 lines (NTSC) and some special purpose cameras supersede this resolution by using higher video frequencies.

Whatever the video source is, the image is digitized, i.e. it is divided into individual pixels (picture elements), the size of which defines the spatial resolution. The gray level (in a black and white image) is an analogue value ranging from the darkest black to the brightest white; this value is digitized by the hardware (digitizer or framegrabber) (Preston 1983; Desai and Reimer 1985) (figure 1). The number of possible gray levels also affects the clarity of the digitized image. The process of digitization is usually performed sequentially and may require several seconds or minutes in low cost digitizers or may be performed in real time, i.e. the video image is digitized at the rate the video source delivers it (40 ms per frame at a 50 Hz video repeat frequency, and 33 ms at 60 Hz). The image is usually stored in a dedicated video RAM (read and write memory) which is part of the digitizer or located in the host computer (Danielsson and Kruse 1980; Steinbach *et al* 1982; Allen and Allen 1983). Thus, after digitization the image is represented as an array of digital numbers. For colour images the 3 basic colours (green, red and cyan) are digitized, either simultaneously or sequentially and stored in different arrays or in the same pixel cell using a specific coding technique (Castillo *et al* 1982). The host computer has access to the stored image in memory and can read and/or write on an individual pixel basis (Kemnitz and Hougardy 1980; Russ and Russ 1984; Mayfield 1984).

Most modern digitizers allow manual or software control of the brightness and contrast of the video signal before digitization. An additional feature, some advanced hardware systems offer, are look-up-tables (LUTs) (table 1). The digitized gray value of each pixel is altered according to a value looked up in a table previously defined (Bryan *et al* 1985). As an example, every value between 0 (darkest black) and 127 (medium gray) is mapped to 0 and every value between 128 and 255 is mapped to 255 (brightest white); by this procedure the contrast is boosted to its maximum represented by only black or white (table 1B). LUTs can also be used to inverse the gray scale (table 1A), to map it in an exponential way, or to expand part of the gray scale (table 1C).

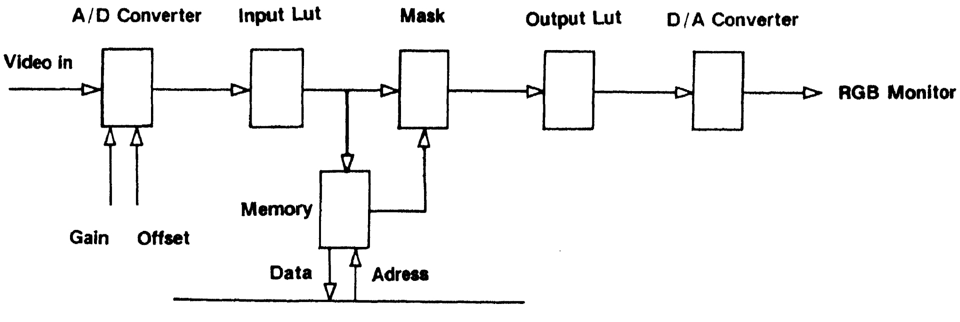

Figure 1. Schematic diagram of video image A/D conversion, input and output LUTs as well as computer access via data and address bus.

Table 1. Use of LUTs to invert the image into its negative (A), to increase the contrast to two levels (B) or to expand the gray level of a small band (124–131) over the whole possible range (0–255) (C). Mapping certain gray level ranges to 3 colors results in pseudocolor representation (D).

	A	B	C	D	
0	255	0	0	0	
1	254	0	0	3	
2	253	0	0	6	Blue
3	252	0	0	9	
4	251	0	0	12	
.	.	.	.	.	
.	.	.	.	.	
.	.	.	.	.	
124	131	0	31	118	
125	130	0	63	121	
126	129	0	95	124	
127	128	255	127	127	Green
128	127	255	159	130	
129	126	255	191	133	
130	125	255	223	136	
131	124	255	255	139	
.	.	.	.	.	
.	.	.	.	.	
.	.	.	.	.	
251	4	255	255	243	
252	3	255	255	246	
253	2	255	255	249	Red
254	1	255	255	252	
255	0	255	255	255	

The digitized image is usually displayed on-line on a suitable monitor (often in parallel to the camera input image before processing). For this purpose the A/D (analogue to digital) conversion is reversed in order to display a digital image; there are also monitors which accept digital input. Before the D/A process and display, the image can again be manipulated using one or more output LUTs (figure 1). The technique of pseudocolour display uses 3 LUTs in parallel to which defined ranges of gray levels are mapped (table 1D). e.g. with 256 possible gray levels in the stored image, all gray numbers ranging from 0 to 85 can be spread out evenly over the range from 0 to 255 on the blue LUT, all between 86 and 170 to 0 to 255 on the green LUT and the remainder to the red LUT. In this example all dark shades are represented by various shades of blue, all bright hues by red colours with increasing brightness and intermediate gray values by green. Any other combination or overlap of ranges can be used for a total of over 16 million colours. Real colour processing requires separate digitization of each of the 3 colour channels on the input side.

There are several hardware approaches which differ in speed, complexity and cost: In dedicated video image analysis the image is processed by a specific hardware designed to manipulate large numbers of pixels at a high frequency. These systems provide the basic techniques to manipulate the image such as brightening, darkening, contrast enhancement or zooming and sometimes even basic analysis techniques such as edge detection, histogram calculation or object identification. In order to enhance speed, often pipeline processors are being used which compute individual pixels in parallel.

Bigger systems allow storing more than one image at a time so that different frames can be compared with each other or substracted from one another which is useful, e.g. to detect a displacement of objects during movement or to eliminate a background (Eichler *et al* 1985).

The graphical representation of the digitized image can be displayed on a video screen, or printed out on a plotter or printer at whatever resolution this device allows. Especially the recent introduction of laser printers boosted the resolution of printed images to about 300 pixels per inch. In addition, the images can be stored on a mass storage device such as a tape or hard disk or floppy disk. However, it should be noted that large amounts of data are handled, which require sufficient storage area, and the transfer may take a considerable time; an image consisting of 512 × 512 pixels takes about 1/4 million bytes of storage area. A monitor system for biological image processing has been described by Lemkin and Lipkin (1980).

3. Image manipulation

Due to the numerical representation of the image in memory, the content of each individual memory cell and thus the image can easily be manipulated in addition to the LUT mapping described in the last section. Increasing or decreasing the brightness requires simply adding or substracting a constant number to or from each pixel, respectively. Likewise, a horizontal and/or vertical ramp can be defined to compensate a linear spatial offset in the original image. The contrast can be enhanced or decreased by suitable multiplication or division of each pixel with a constant. Thresholding is another useful technique to set anything below a predefined value to zero or everything above a given value to the maximal level in order to remove noise in the background.

More advanced mathematical techniques such as Laplace or Sobel algorithms are based on calculations involving a 3 × 3 (or higher) matrix of pixels. Each pixel in the derived image is calculated from the original value and that of its neighbours. As an example, during Laplace filtering, each pixel is multiplied by a specific number (kernel), which is 8 for the most common algorithm, and the values of all its surrounding 8 neighbours are substracted from this value (table 2a, b, c).

$$\begin{matrix} -1 & -1 & -1 \\ -1 & 8 & -1 \\ -1 & -1 & -1 \end{matrix}$$

The visible result of this operation is a reduction in brightness of all areas with similar grey levels while sharp boundaries show up as bright lines. Similarly, averaging can be achieved by replacing each pixel E′ by the geometric mean value from the original value E and that of its 8 neighbours.

$$\begin{matrix} A & B & C \\ D & E & F \\ G & H & I \end{matrix}$$

$E' = (A+B+C+D+E+F+G+H+I)/9.$

This technique smoothes sharp boundaries and reduces noise (Julez and Harmon 1984).

Table 2a. Original pixel values in part of a digitized image.

60	52	61	63	75	71	68	72	78	81	79	84	85
61	64	140	72	75	71	79	80	85	81	84	89	92
58	61	64	67	80	70	72	75	78	80	78	82	85
64	71	72	75	71	69	220	231	225	228	229	231	230
65	70	58	73	75	230	229	228	234	227	230	232	218
69	72	75	73	225	247	230	231	235	237	228	229	240
71	75	72	74	68	242	250	231	232	232	228	237	239
79	69	72	75	76	71	228	229	235	235	236	228	227
60	63	65	71	72	72	228	229	230	225	230	230	225
63	70	68	72	74	73	230	229	231	234	234	230	228

Table 2b. Noise reduction and smoothing by averaging over a 3 × 3 matrix in table 2a.

60	52	61	63	75	71	68	72	78	81	79	84	85
61	69	72	77	72	73	73	76	79	80	82	84	92
58	73	76	80	72	90	107	127	129	130	131	133	85
64	65	68	71	90	124	158	177	178	179	180	179	230
65	68	71	89	126	177	213	229	231	230	230	230	218
69	70	71	88	145	200	235	233	232	231	231	231	240
71	73	73	90	128	182	218	233	233	233	232	232	239
79	70	71	72	91	145	198	232	231	231	231	231	227
60	68	69	72	73	125	177	230	231	232	231	230	225
63	70	68	72	74	73	230	229	231	234	234	230	228

Table 2c. Result of Laplace filtering with a kernel of 8 using values in table 2a.

60	52	61	63	75	71	68	72	78	81	79	84	85
61	0	255	0	31	0	53	33	55	5	18	43	92
58	0	0	0	70	0	0	0	0	0	0	0	85
64	56	37	40	0	0	255	255	255	255	255	255	230
65	14	0	0	0	255	146	0	30	0	0	21	218
69	21	33	0	255	255	0	0	28	50	0	0	240
71	21	0	0	0	255	255	0	0	0	0	41	239
79	0	12	30	0	0	255	0	37	32	43	0	227
60	0	0	0	0	0	255	0	0	0	0	2	225
63	70	68	72	74	73	230	229	231	234	234	230	228

Zooming is achieved by spreading out each pixel over several ones which magnifies the image, though it does not increase the resolution (Hainfeld *et al* 1982). Most systems allow only zooming by integer numbers. Gradual zooming requires interpolation between pixels. In systems which display only part of the image on screen, scrolling and panning in horizontal and vertical directions is useful. At this point we suspend the discussion of object detection in the image though it is sometimes realized using hard-wirded procedures.

4. Electrophoresis analysis

Single dimension electrophoresis and chromatography (Mancini *et al* 1978; Ford-Holevinski *et al* 1983) can easily be evaluated using computer-assisted video analysis (Gray *et al* 1984). The gel is mounted on a backlit screen such as a light box with an even irradiation over the entire area (figure 2). Similarly, photographic positives or

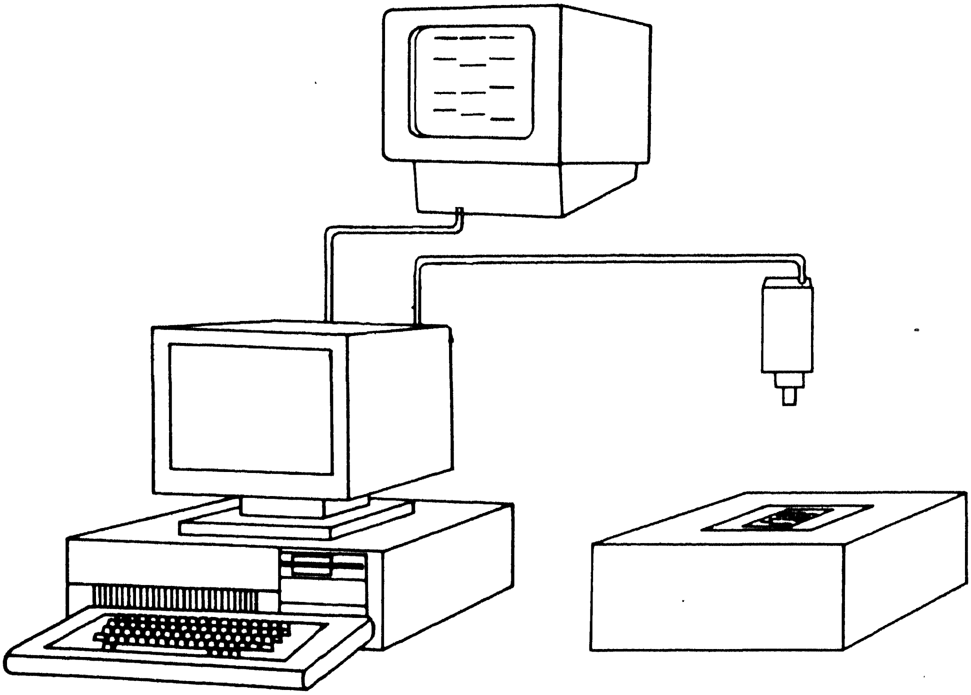

Figure 2. Image analysis system to scan electrophoresis gels consisting of an AT microcomputer, a b/w video camera, a monitor and a light box.

negatives of the original gel or autoradiographs (Elder *et al* 1986) can be used as the basis for the scan. The software is designed to scan across the bands, such as stained proteins, at a predefined position and record the gray levels as it advances (Häder and Truß 1987) (figure 3). If accurate quantification is required, the camera has to represent grey levels in a linear fashion which is not always the case due to the automatic gain adjustment built into most cameras, which needs to be disabled for this purpose. In addition, the experimenter has to ensure, that both extremes in brightness (background and bands) do not exceed the digitization range. For more accuracy the gel can be scanned several times at the same or different positions and the results are averaged. The recorded values represent a faithful image of the spatial arrangement and the absorption values of the bands.

The spatial resolution depends on the optics used with the camera and can easily be boosted using either a macro objective or even a microscope objective; by this measure very high resolutions can be realized easily which is an advantage over conventional densitometers which usually operate with a fixed resolution (Jarvis 1981). Likewise, the products of more recent techniques such as Northern or Western blots can be scanned using similar software.

The next step after the initial scan could involve pattern recognition which is an application, e.g., for DNA sequencing gels (Mount and Conrad 1986; Elder *et al* 1986). Since routine analysis of sequencing gels is a rather cumbersome task which is prone to error, this application is a useful tool (Cannon 1987).

Two-dimensional gels can also be easily analyzed by extending the scan to two

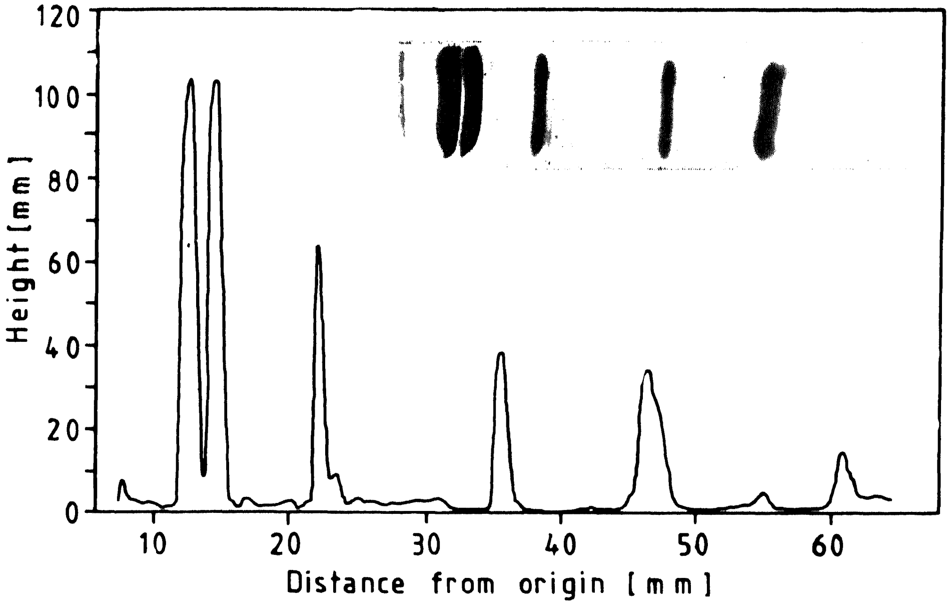

Figure 3. Digitized video scan of a protein gel (inset) of the ATPase isolated from *Wolinella succinogenes* (after Häder and Truß,1987).

dimensions (Spragg *et al* 1983; Tyson and Haralick 1986). This technique lends itself to automated substance identification when the digitized pattern is compared to prerecorded patterns of known substances. By using an appropriate software, even distortions in either or both directions can be eliminated if known markers are used to calibrate the system.

A recent application is the analysis of electrophoresis gels with DNA stained with fluorescent substances, such as ethidium bromide. The gel is placed over UV-emitting lamps and the fluorescence is recorded by the camera (Häder and Truß 1987) (figure 4). For this purpose a sensitive video camera is required to detect even weak fluorescence. If necessary, an electronic image intensifier can be employed.

A different design of a computerized microspectrophotometer was developed to perform sequential measurements on cells in order to determine the nuclear DNA content or grain counting from autoradiographs. The light from the microscope was carried by optical fibre bundles to the photomultipliers (Neely *et al* 1984).

Finally, the recorded data can be represented graphically or numerically or stored for further mathematical or statistical analysis. Similarly, protein-specific fluorescent probes used in thin-layer chromatography have been analyzed using quantitative image analysis (Rees *et al* 1985).

5. Object counting and area analysis

Cell counting is a task which is routinely performed in many medical and biological laboratories. The same approach can be used to count colonies, organisms or other macroscopical objects such as leaves (Kincaid and Schneider 1983). Several systems have been developed to determine cell densities (Häder and Griebenow 1987). The

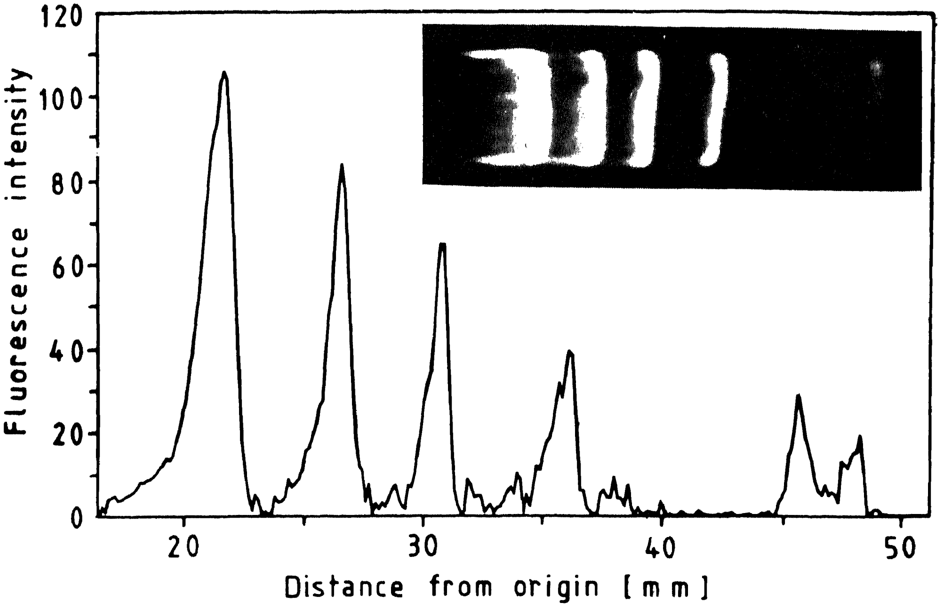

Figure 4. Digitized video scan of the fluorescent ethidium bromide stained phage lambda DNA bands (inset) on an agarose gel (after Häder and Truß 1987).

cell suspension is filled into a cuvette of known depth. Motile cells do not need to be immobilized for this purpose if real time digitization is used. The image is scanned line by line until an object is found which exceeds the background by a predefined threshold (figure 5A). Now a filling algorithm is employed to determine which of the adjacent pixels belong to the organism. All identified pixels are set to a defined gray level below the threshold (figure 5B). The algorithm continues to fill the object in shells around the initial point until all connected pixels have been identified. The number of pixels found represent the area of the cell (or other object) (Häder 1987a; Kim *et al* 1987). Subsequently, scanning is resumed from the point where the filling algorithm was started until the whole frame has been analyzed.

One problem occurs when objects are in direct contact (Erhardt *et al* 1980). Previous systems calculated the size of the object and rejected it when it was outside the predefined boundaries. However, in dense suspensions a large proportion of valid objects may be rejected by this algorithm. In order to overcome this situation, an erosion technique is applied: Shells of one pixel wide around the circumference of each object are set to a specific gray value. This process is repeated until connecting areas are separated at the isthmus. Subsequently, the shells are filled in again to their original value provided that a gap of at least one pixel wide is left between adjacent objects. Alternatively, objects can be separated manually before analysis using the cursor controls, a mouse or digitizing tablet or a touch sensitive screen.

The process of counting and area determination can be repeated on the same sample after renewed digitization or on a different sample for averaging purposes. The numerical results can be used to calculate histograms of the area distribution. Several mathematical operations can be used to analyze the object such as determining the largest diameters or finding the center of gravity (figure 6).

The approach of cell counting was recently applied to evaluate the cell densities in

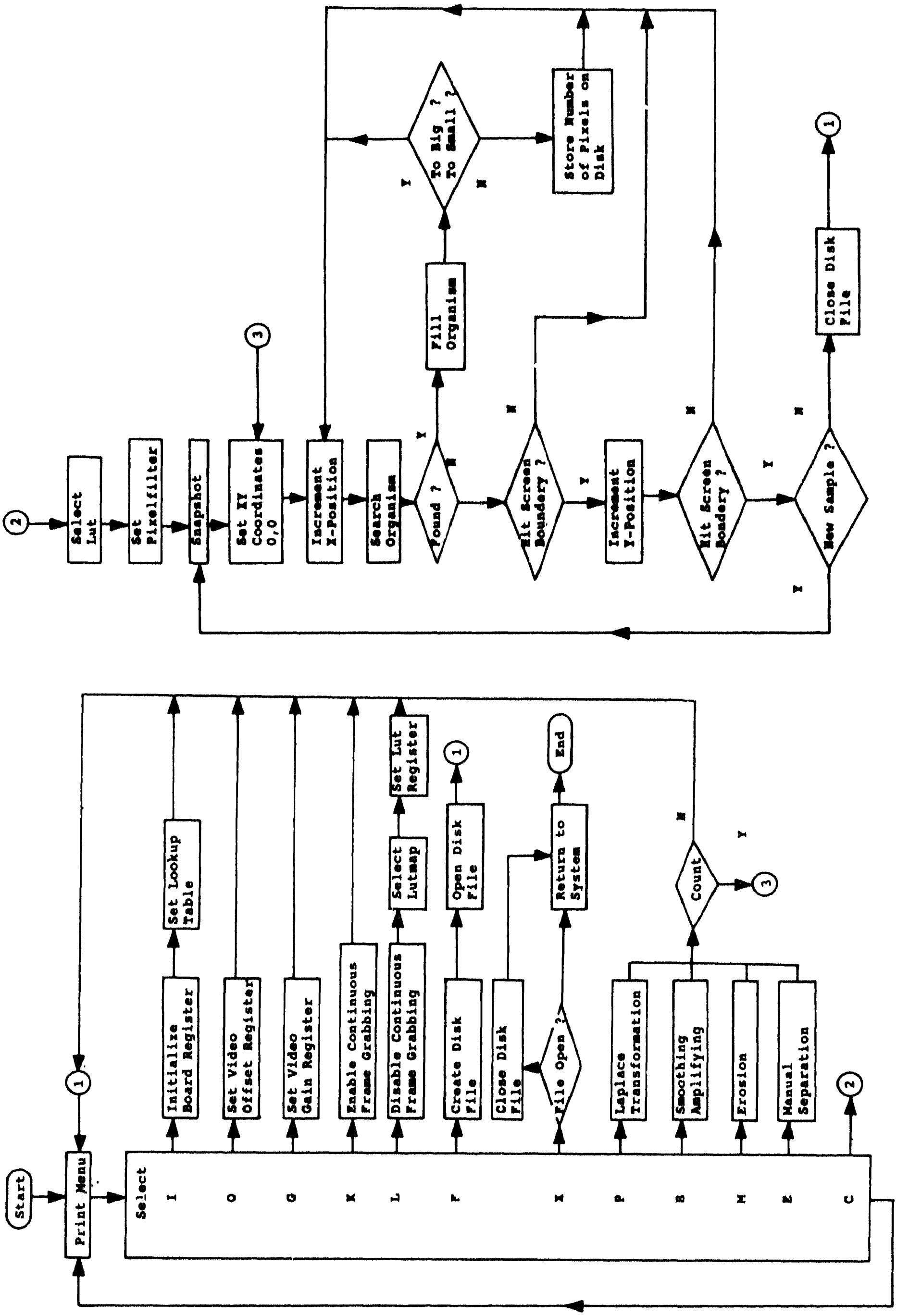

Figure 5. Flowchart of the algorithm to detect an organism in an image and calculate the area using a filling algorithm. In addition, a number of housekeeping routines such as disk file handling are incorporated.

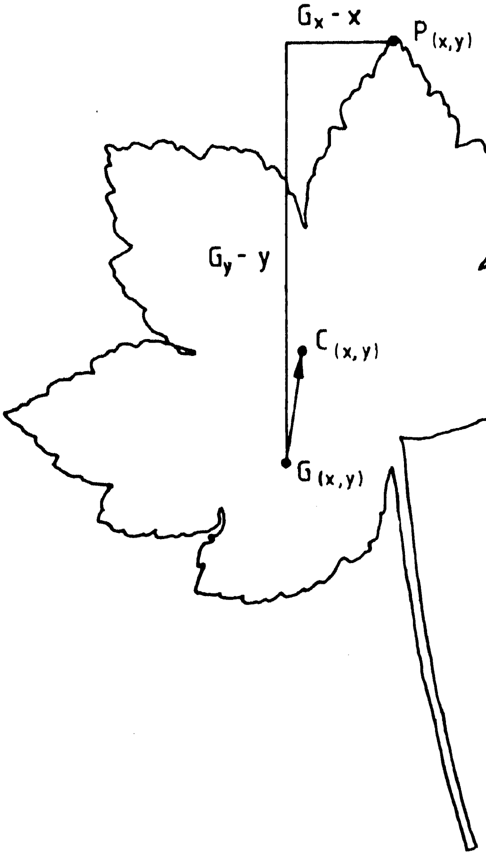

Figure 6. Calculation of the center of gravity in an irregular shaped object such as a leaf (after Häder 1987a).

samples of *Euglena* populations filled in a column submerged in a pond (Häder and Griebenow 1988) (figure 7). Samples were taken at regular time intervals by a peristaltic pump which could handle 18 samples in parallel from 18 outlets evenly spaced along the length of the column. By this approach the distribution of the population in dependence of light and gravity could be determined in a time resolved fashion and documented in linear histograms (figure 8).

Another application is the determination of the size distribution of tapeworm larvae taken from infected mice. After removal from the host the larvae were suspended in a petri dish. Shadowgraphs were taken by projecting the image onto extra hard photographic paper (Häder *et al* 1980); these prints were subjected to image analysis. Size distribution was documented by linear histograms.

6. Cell analysis

6.1 *Cell characterization and identification*

Early in the development of image analysis researchers found interest in the digitization of cellular structures (Holmquist *et al* 1981; Smith 1982; Skarnulis 1982;

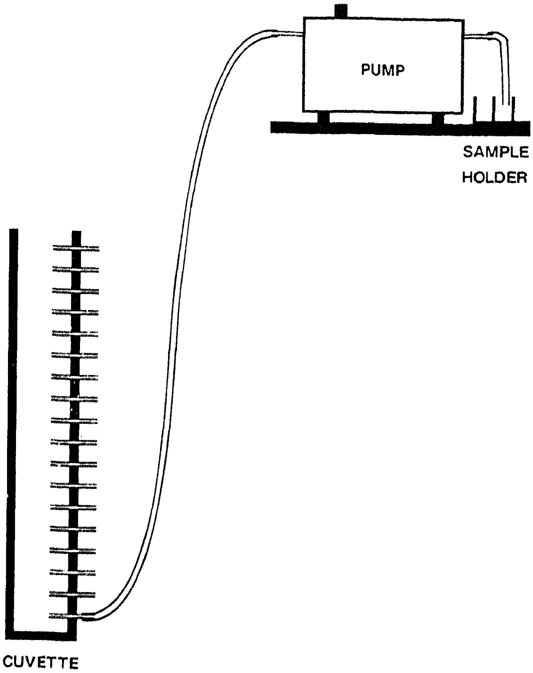

Figure 7. Schematic diagram of a plexiglass column with 18 outlets from which 18 samples can be drawn in parallel using a peristaltic pump (after Häder and Griebenow 1988).

Spring 1983). Both light microscopy (Walter and Berns 1981; Inoue 1981; Caldwell 1985; Wollmer 1987) and electron microscopy (Gravekamp *et al* 1982; Kirkland 1982; Boyes *et al* 1982; Gronsky 1988) are important fields for image enhancement and analysis and even holograms have been used as image sources (Lin and Cowley 1986). Especially in medical samples it is a declared goal to develop automated image analysis systems which allow the recognition and identification of specific features (Rodenacker *et al* 1980; De Paz *et al* 1986) in order to reliably determine e.g. quantitative distributions of blood cells or sediments (Koss *et al* 1983; Preston 1986) and to identify abnormal cells such as cancer cells (Preston and Dekker 1980; Preston 1981; Preston *et al* 1984; Kaufman *et al* 1987; Wittekind and Schulte 1987). Reliable cell identification requires a high spatial resolution of > 10 pixel/μm (Gunzer *et al* 1987). A system based on a 2-dimensional microdensitometer has been proposed to analyze electron micrographs (Shaw *et al* 1981). In addition to morphometric methods, image analysis and reconstruction is used for 3-dimensional reconstruction of biological structures (Nierzwicki-Bauer *et al* 1983; Gras 1984; Jimenez *et al* 1986).

Another application is the automated analysis of cells in intact tissues to determine the effects of damage by trace gases (Omasa *et al* 1982, 1983, 1985). The movements of stomates have been studied using image analysis in order to determine the effects of SO_2 or NO_2 on the plant (Omasa *et al* 1981).

6.2 *Organelles and protein analysis*

Not only tissues and single cells are analyzed but morphometric studies are also used

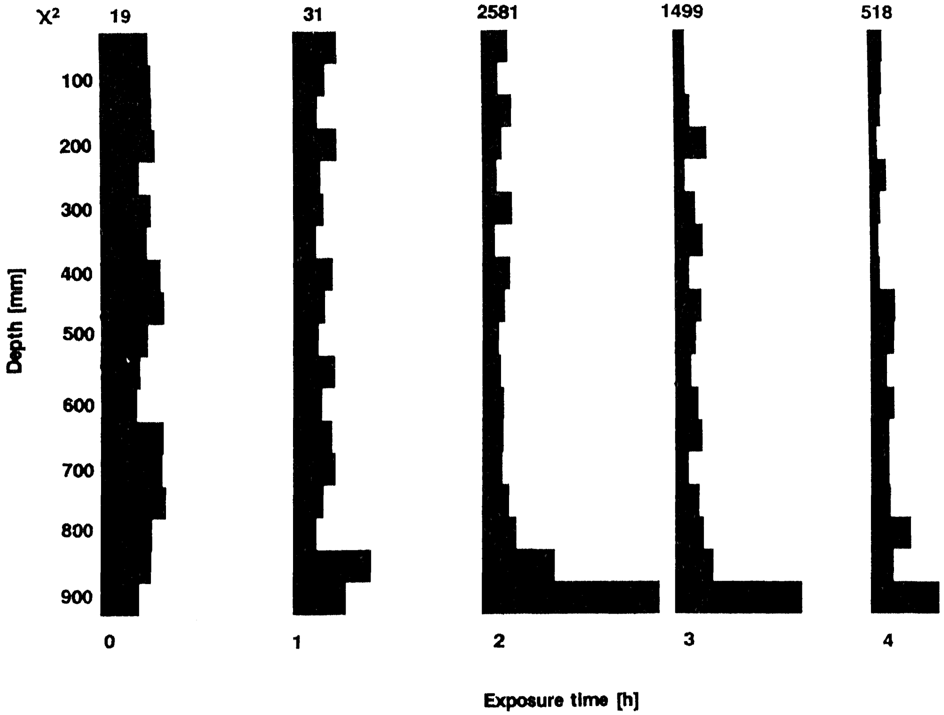

Figure 8. Linear histograms of the cell density distribution in the column after increasing periods of exposure to solar radiation. The numbers above the histograms indicate the Chi square values (after Häder and Griebenow 1988).

to reveal intracellular structures (Rodenacker *et al* 1980; Iwama and Eguchi 1986) such as the cytoskeleton (Weiss 1986), the chromatin structure (Chegini *et al* 1981) or peroxysomes (Beier and Fahimi 1986). Even macromolecular structures such as proteins have been analyzed (Boekema *et al* 1986; Gogol *et al* 1987). In addition to the techniques discussed above, the method of image averaging is used for the reconstruction of subcellular structures. Electron microscopic images of particles such as ribosomes are digitized and superimposed electronically after proper alignment so that the resulting image is the average over many individual samples (Crowther and Klug 1975; Kuntz and Crippen 1980). This technique removes noise and visualizes images of structures near the resolution of the instruments. Using the SEMPER image analysis system, even the 3-dimensional structure of a membrane pore could be visualized in *Escherichia coli* (Dorset *et al* 1984) as well as the structure of the flagellar hrook in *Caulobacter crescentus* (Wagenknecht and De Rosier 1981).

6.3 *Fluorescence and radioactive labelling*

Often fluorescence from living cells does not provide high intensities. In addition, the fluorescence decays over time due to fast bleaching of the fluorescent dyes used. Digital image storage and electronic video amplification are techniques to visualize low energy fluorescence images (Barrows *et al* 1984). The chlorophyll fluorescence has been analysed as a diagnostic tool to determine the effect of pollutants on the photosynthetic apparatus in the intact leaf (Omasa *et al* 1987).

Fluorescence can also be used to make physiological processes within the cell visible. Calcium pools inside the cell can be observed by using an intracellular fluorescent dye such as aequorin or Fura-2 (Williams *et al* 1985; Conner 1986); similarly the membrane electrical potential (Gross *et al* 1986) or the cytoplasmic pH (Tanasugarn *et al* 1984) can be visualized. Even the intracellular motion of individual molecules has been measured (Webb and Gross 1986).

Likewise, computerized pattern recognition has been used for grain counting in high resolution autoradiographs with low grain densities (Schellart *et al* 1986).

7. Organism tracking

Unlike the analysis of static images, tracking the movement (Davenport *et al* 1970; Davenport 1973; Coates *et al* 1985; Rikmenspoel and Isles 1985; Burton *et al* 1986) or growth of an organism (Gordon *et al* 1984; Omasa and Onoe 1984; Jaffe *et al* 1985; Omasa and Aiga 1987) requires the comparison of a series of subsequent frames (Mineyuki *et al* 1983; Allen 1985; Dow *et al* 1987; Amos 1987). The objects can range from individual cells such as flagellates or ciliates (Häder 1985b; Mikolajczyk *et al* 1985, 1986; Häder 1986, 1987b; Häder *et al* 1986, 1987, 1988) to multicellular organisms (Dusenbery 1985a, b). Several techniques have been developed to analyze individual tracks of organisms. In one approach subsequent images of the object are substracted from each other which outlines the increments in movement between the frames. These area increments between subsequent frames are connected to form a track as wide as the organism. After sufficiently long tracks have been calculated they are reduced to fine lines by a process called "skeletoning": pixels are removed from both sides of the wide track until a line one pixel wide is left.

Depending on the time requirements, the organisms are tracked either on-line (Katz *et al* 1985) in real time as they move along their path or the tracks are recorded on a suitable video storage medium and are analyzed subsequently (Glazzard *et al* 1983; Herman and Albertini 1984). The first approach can only be accomplished by using Assembly Language programming in order to avoid the sometimes considerable computation overhead of higher languages. Since the off-line approach can be done at a much slower time scale, programs for this purpose have been written in higher languages such as Fortran or C, sometimes using Assembly Language subroutines for the basic image acquisition subroutines.

7.1 *Off-line tracking of recorded movements*

Early semiautomatic track analysis was performed by copying track vectors of cells on a transparent overlay from a large videoscreen (Häder *et al* 1981). The x- and y-coordinates of the beginning and end of each individual track were entered into a computer by using a bit pad digitizer or a mechanical device (Lipson and Häder 1984). The computer then calculated the angular deviation from the stimulus direction, such as the light beam.

In a more recent approach, off-line tracking has been used to study the movements of individual cells of the flagellate, *Euglena gracilis*, in lateral light beams. For this purpose the cell suspensions were transferred into a suitable cuvette on the stage of a microscope and subjected to irradiation from the actinic light beam. The movements were continuously recorded on a high quality video tape recorder (Gualtieri *et al* 1985).

The subsequent analysis was based on a computer which controlled the video tape recorder and thus allowed to request the next frame as soon as the analysis of the previous frame was finished. The position of each organism in the focal plane was determined and recorded for subsequent frames. Finally, the recorded tracks were analyzed for deviation from the light direction.

A similar approach was used by a system which allowed to follow up to 25 individual organisms simultaneously (Dusenbery 1985a, b). Likewise, recordings of ciliary and flagellar beat were analyzed after digitization (Sanderson and Dirksen 1985; Baba and Mogami 1985; Omoto and Brokaw 1985). The phototactic orientation of the slime mould *Physarum* was analyzed by automated measurement of the oscillatory changes in thickness of the plasmodium (Mori *et al* 1986). Intracellular movements were analyzed by visualizing colloidal gold particles using tracking algorithms to follow movement of the markers in video recordings (Geerts *et al* 1987). Likewise, the movement of marker particles was used to analyze the growth pattern of *Phycomyces* sporangiophores (Gamow *et al* 1986). Using a bugwatcher, video recordings of zooplankton populations were analyzed to study the behaviour to simulated bioluminescence (Buskey and Swift 1985).

7.2 *Real time analysis of movement direction*

Real time analysis of movement tracks requires a fast hardware system and efficient software approaches since the video repeat rate allows only 33 or 40 ms between frames for calculation (figure 9).

One approach is based on the strategy to follow a randomly selected cell for a predefined period of time (Häder 1981, 1985a; Häder and Lebert 1985). The algorithm starts by scanning a digitized image from a random position until it detects an organism. For elongated organisms it is essential to scan both horizontally and vertically at random in order not to discriminate against organisms moving parallel to the scan line.

After an organism has been detected, its outline is determined using standard edge algorithms (Grant and Reid 1981; Berns and Berns 1982). At this point a first safety check is performed to determine if the object is in the predefined range of sizes to discriminate it from debris and to exclude objects in contact with others. Subsequently the centroid (center of gravity) is calculated and stored. Starting from this centroid, the position of the same organism is determined in the next digitized frame. This process is repeated a predefined number of times. A movement vector is calculated from the first and last centroids and stored as an angular deviation from the stimulus direction in a disk file for subsequent mathematical and statistical evaluation, provided the organism has moved at a speed within preselected limits to exclude immotile organisms or movement vectors faked by convection currents or sedimentation rather than by active swimming.

Analysis of microscopic organisms, especially when only weakly absorbing such as ciliates or colourless flagellates, is greatly facilitated by using dark field irradiation. In the case of photosensitive organisms it is mandatory to use an infrared monitoring

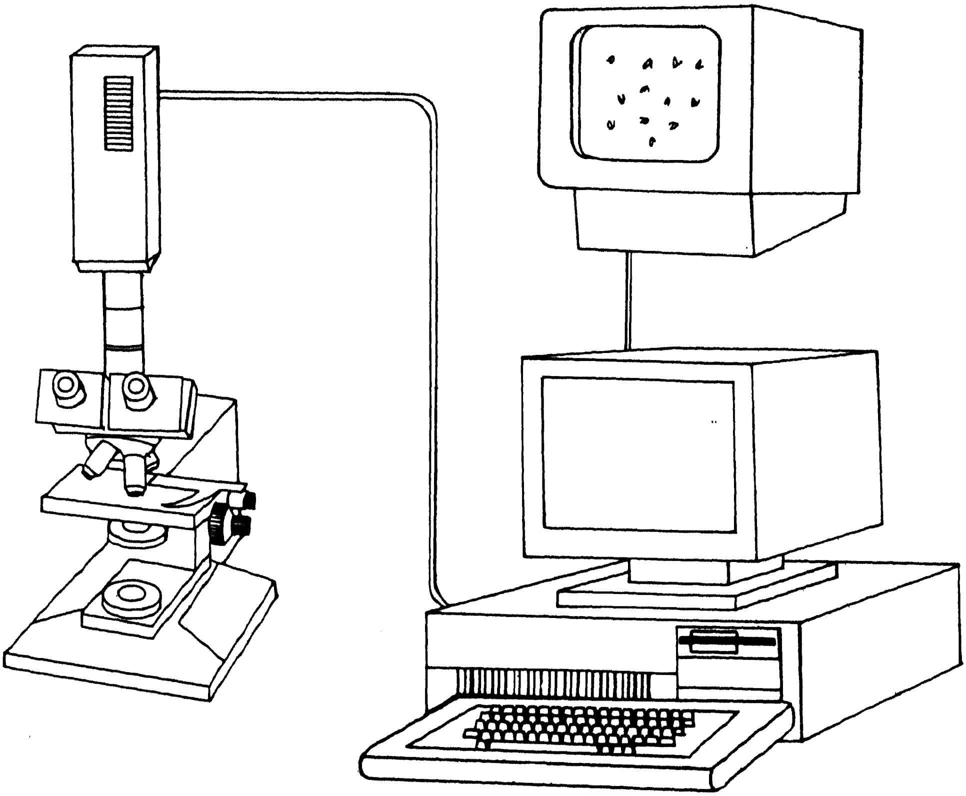

Figure 9. Image analysis system to follow individual motile microorganisms consisting of an AT microcomputer, a b/w video camera on top of a microscope and a monitor.

light beam, which does not affect the photosensory behaviour of the organisms and does also not stimulate the photosynthetic apparatus, since the oxygen produced by organisms in the field irradiated by the monitoring beam is known to induce directed movements in a number of organisms (Häder 1979).

The raw data (angular deviation from the stimulus direction) are used to construct histograms using 64 sectors with a width of 5·6° each (Häder and Lipson 1986) (figure 10A, B). The histograms are graphically presented in a circular diagram. In addition, directional statistics is used to determine the degree of orientation based on the Rayleigh test (Batschelet 1965, 1981; Mardia 1972):

$$\bar{r}=\frac{\sqrt{(\Sigma \sin \alpha)^2+(\Sigma \cos \alpha)^2}}{n}$$

An $\bar{r}$-value in the vicinity of 0 indicates that the organisms are more or less randomly distributed and an $\bar{r}$-value close to 1 indicates that most organisms are oriented in the same direction with little deviation from the preferred mean direction. However, the Rayleigh test is not valid for multimodal distributions and does not indicate the direction of movement. For this purpose Fast Fourier Analysis has been employed (Häder and Lipson 1986). The highest Fourier coefficient indicates

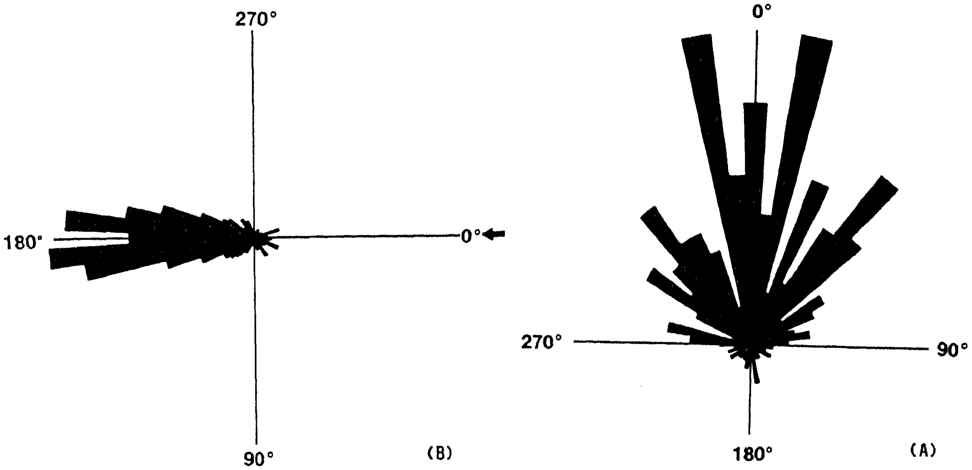

Figure 10. Circular histograms of (**A**) *Cryptomonas* negative gravitaxis and (**B**) *Euglena* negative phototaxis (after Rhiel *et al* 1988; Häder and Häder 1988b).

the number of modes and the corresponding angular component indicates the preferred main angular orientation (figure 11). Fourier analysis has also been used to determine periodicities in electron micrographs (Harms *et al* 1981; Squire *et al* 1986).

Similar algorithms have been used to analyze the eye movements (Hall 1983) and facial movements (Dowideit *et al* 1983) in high resolution.

7.3 *Evaluation of motility*

Another important application of automatic image analysis is determination of motility. A similar approach to the one described in the last section has been used for this purpose (Häder and Häder 1988a, b). The cells move in a cuvette wide enough to allow unrestricted movement. The image is recorded by a high resolution CCD camera mounted on a conventional light microscope equipped with infrared dark field irradiation. The position of randomly chosen cells is determined as described before in subsequent frames and the linear displacement is stored for further calculation together with the corresponding time interval derived from the computer's internal hardware clock which is accurate to a one hundredth second.

Subsequent programs use the raw data to determine the individual speed of each recorded organism and to construct linear histograms showing the velocity distribution of the population. In addition, the percentage of motile cells in the population is calculated. A graphic program is utilized to compute the block sizes for graphical representation of the velocity histograms on a laser printer at a resolution of 300 dots per inch (figure 12).

7.4 *Analysis of track parameters*

Another important aspect of track analysis is the extraction of key parameters from the paths of moving organisms. One example is the determination of photophobic responses. Many photosensitive motile microorganisms respond to sudden changes

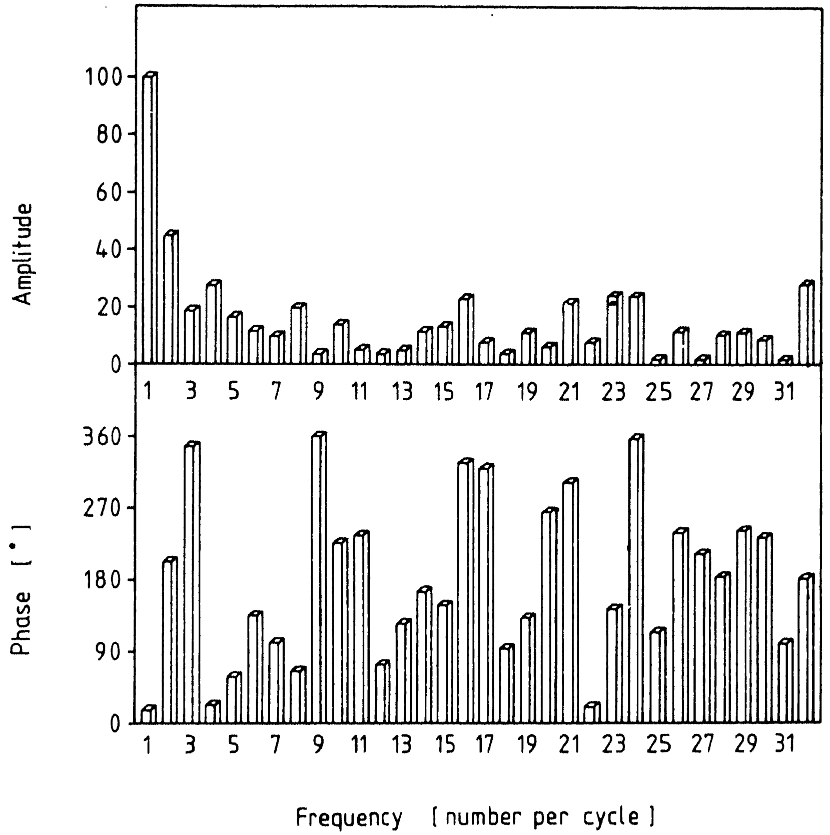

Figure 11. Fourier analysis of a histogram showing positive phototaxis of *Dictyostelium* pseudoplasmodia (after Häder and Lipson 1986).

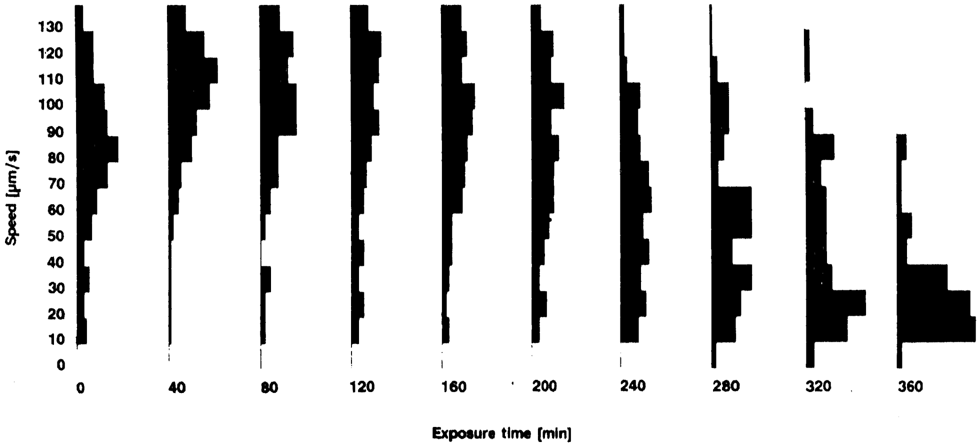

Figure 12. Linear histograms of the velocity of *Euglena gracilis* after increasing exposure to solar radiation (after Häder and Häder 1988b).

in the fluence rate by a transient change in their movement pattern such as a turn or a reversal of movement (Häder and Burkart 1982; Doughty and Diehn 1984; Melkonian *et al* 1986). The archaebacterium, *Halobacterium halobium* swims parallel to its long cell axis and reverses its direction of movement at regular time intervals (Schimz and Hildebrand 1985). An increase in the actinic green/orange (attractant) light or a decrease in UV/blue (repellent) light suspends the next autonomous reversal by several seconds (Wagner 1984). In contrast, a decrease in the attractant or an increase in the repellent stimulus induces a premature reversal. In order to automate the behaviour analysis, the exact reversal points need to be determined along the path (Takahashi and Kobatake 1982; Spudich 1985). Alternatively, a cell can be tethered by a flagellum to a glass support so that the cell moves in circles clockwise and counterclockwise. In this case, the change in angular velocity needs to be determined by the software.

Cells moving along their paths change directions due to two reasons in addition to responses to sensory stimuli. Changes in the path can be induced by collisions with other cells or obstacles or by capricious turns not initiated by obvious external factors. In order to determine the behavior of a population, these changes need to be extracted from the path (Kessler 1985, 1986). From this information important parameters such as the free swimming path or the turning frequency can be determined.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 4, August 1988, pp. 251–255.
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Anther development in *Xylopia nigricans*

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MS received 14 September 1987; revised 14 March 1988

Abstract. In the anther primordium of *Xylopia nigricans*, the archesporium differentiates discontinuously with intervening sterile tissue. Each archesporial cell divides periclinally and develops into a micro-sporangium with a single microspore mother cell. Thus the anther becomes multisporangiate. The tapetal cells are uninucleate. Cytokinesis of the microspore mother cell is by successive cell plate formation. The pollen development is traced and the significance of the development is discussed.

Keywords. *Xylopia*; Annonaceae embryology; anther development; multisporangiate anther; microsporogenesis.

1. Introduction

In most angiosperms, the young microsporangium has a single mass of spore mother cells. Some deviate from this and form sterile transverse septa which is a constant feature in some taxa while in others it is sporadic. Many members of the Annonaceae show septa (Larsten 1971) and the septal development has been studied only in *Cananga odorata* (Periasamy and Swamy 1959) and *Annona squamosa* (Periasamy and Kandasamy 1981). Schnarf (1931) reported sterile partitions in *Xylopia nigricans* of the family and in the present study, the ontogeny and morphology of the partitions and microsporogenesis in this plant are described.

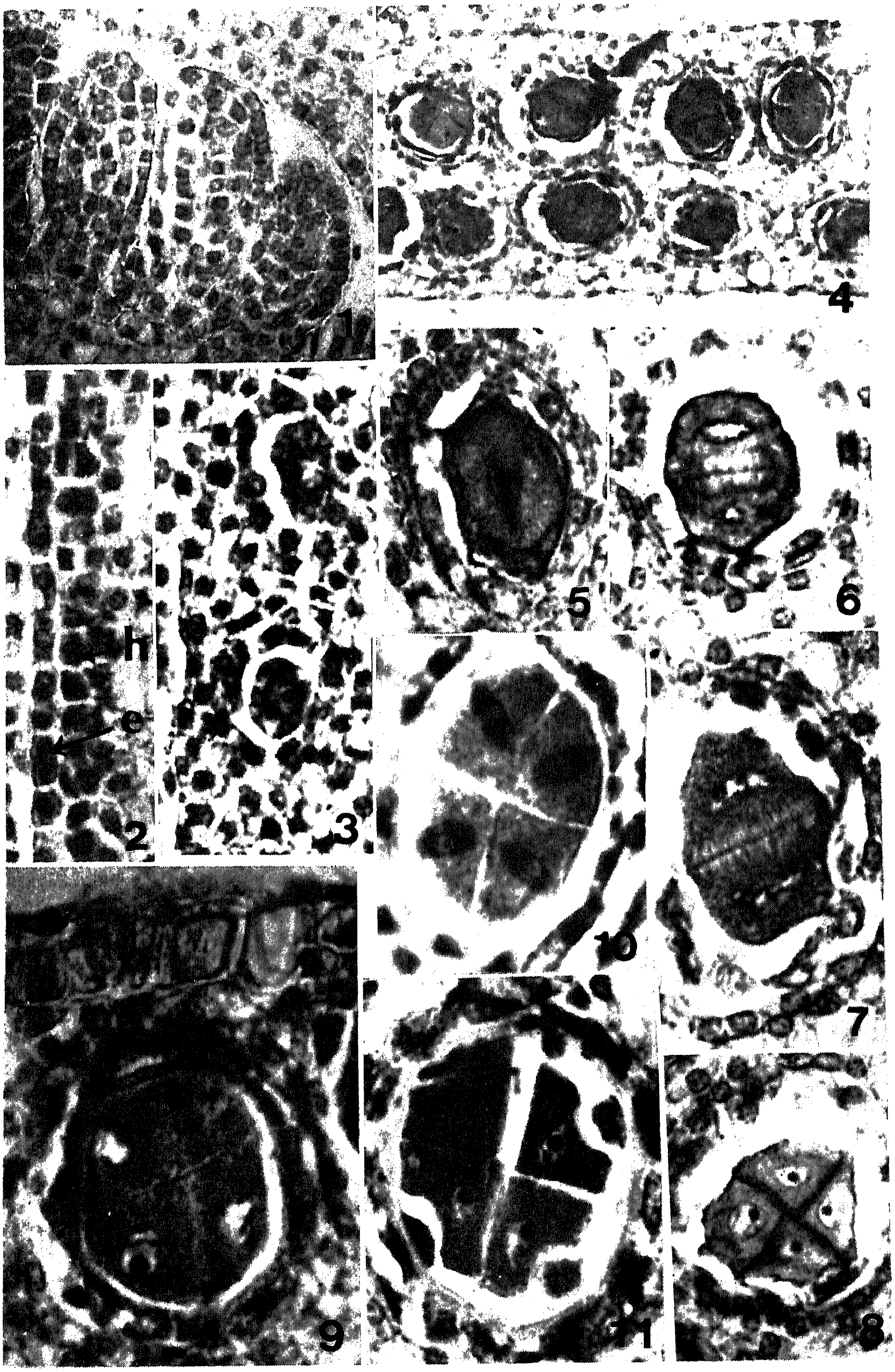
2. Materials and methods

Flower buds of *Xylopia nigricans* Hk.f.et.Th. were collected from Alagar hills, Madurai district. The perianth was removed from mature flower buds and the material fixed in FAA. Dehydration was carried out in ethanol-trichloroethylene series and infiltrated and embedded in paraffin wax. Sections taken at a thickness of 10 μm were stained with toluidine blue or haematoxylin and erythrosin following the method of eliminating ethanol series (Periasamy 1967).

3. Results

3.1 *Anther development*

The staminal primordium initially comprises a mass of undifferentiated meristematic cells (figure 1). It becomes 4 angled and a single row of hypodermal cells become distinct in each corner of the angle. These hypodermal cells do not exhibit any morphological or cytological differences but their division activity is not synchronous. While some cells located at intervals remain undivided, others between them



Figures 1–11.

divide in different planes (figure 2) and the derivatives become sterile tissue (figure 3). The undivided cells divide at a later stage periclinally and each gives rise to an outer primary parietal cell and an inner primary sporogenous cell.

The primary parietal cell divides further to form 3 wall layers (figures 3 and 4). The primary sporogenous cells do not divide further but enlarge and become the microspore mother cells, which are separated from each other by the intervening sterile tissue (figures 3 and 4). The anther thus becomes multisporangiate with 4 rows of microsporangia each of which contains only one microspore mother cell. The maximum number of microsporangia observed in one row is 13 and hence each anther may have a total of 50–60 microsporangia.

The innermost wall layer as well as those of the sterile septa and the connective tissue that adjoin the microspore mother cell differentiate into the tapetum around the microspore mother cell (figures 5 and 6). The tapetum is single layered, secretory and remains uninucleate throughout (figures 5–7); its cells get separated from each other after the second division of the microspore mother cell and subsequently degenerate (figures 8–10).

The hypodermal layer of the sporangial wall as well as those of the sterile septa differentiate into the endothecium with the characteristic fibrous thickening (figure 9). The middle layers persist even after the first division of the microspore (figure 9).

3.2 *Microsporogenesis*

The first meiotic division (figure 5) usually takes place at right angles to the longitudinal axis of the anther but it may also be parallel. This is followed by a distinct phragmoplast that forms a centrifugally expanding cell plate (figure 6), which divides the cell into two (figure 7). The second division at right angles to the first but in the same plane, divides the cell into 4 by centrifugal cell plates to form an isobilateral tetrad (figure 8).

3.3 *Microspore*

The microspores do not separate and the tetrad is enclosed by a common wall without the development of individual wall for each spore (figures 8 and 9). The first division of the united microspores takes place simultaneously at the respective distal poles (figure 10). Thus each microsporangium develops 4, two celled pollen, each with a small generative cell and a large vegetative cell (figure 11), and it is shed as such.

Figures 1–11. *Xylopia nigricans*. **1.** LS of 3 anther primordia (×350). **2.** LS of anther primordium showing the epidermis, hypodermal archesporial (undivided) and non-archesporial (divided) cells (×550). **3.** LS of anther showing two microspore mother cells, intervening septum and wall layers (×250). **4.** LS of multisporangiate anther showing two rows of sporangia out of the 4 (×300). **5.** Metaphase of first meiotic division of the microspore mother cell with surrounding uninucleate tapetum (×450). **6 and 7.** Two stages of cell plate formation and phragmoplast after first meiotic division (**6.** ×450; **7.** ×500). **8.** Isobilateral tetrad immediately after completion of cytokinesis (×420). **9.** Tetrad just before first division of microspore with distally placed nuclei; note endothecium of anther wall (×550). **10.** Anaphase of the simultaneous first division of the microspores of a tetrad (×550). **11.** Two celled pollen tetrad surrounded by a thick common wall; note the absence of individual walls (×550). (e, epidermis; h, hypodermis).

4. Discussion

The behaviour of the archesporium and the sporogenous tissue and the formation of sterile septa in the anthers of the Annonaceae, indicate that in this family of the Ranales, microsporangium development presents a gamut of variation that would lead to the development pattern prevalent in most angiosperms (Periasamy and Kandasamy 1981). Variations reported in the microsporangium, including the present study, are presented in table 1.

In *Annona squamosa* (Periasamy and Kandasamy 1981), unlike in *Xylopia nigricans*, all the hypodermal cells undergo the first periclinal division almost simultaneously and alternate inner derivatives, equivalent to primary sporogenous cells, become sterilized to form septa. In *Cananga odorata* (Periasamy and Swamy 1959) the sterilization takes place at a much later stage when the sporogenous cells have enlarged considerably and a few (1–3) septa are formed.

Table 1. Variations in the anther and microsporangium of the Annonaceae.

Character	Variations	Taxa*	References
No. of sporangia per anther	Four	1–5,6,7, 10,11.	Asana and Adatia (1947)
	Four to eight	6	Juliano (1935)
	Numerous	12	Lecomte (1896)
Nature of sporogenous tissue	Massive	4,7,8.	Locke (1936)
	Biseriate	5	Parulekar (1970)
	Uniseriate with occasional partitions	6	Periasamy and Kandasamy (1981)
	Uniseriate with sterile partition alternating with each spore mother cell	1,2,3,9.	Periasamy and Swamy (1959) Sastri (1957)
	Single celled	12.	Schnarf (1931)
Type of cytokinesis	Simultaneous centripetal furrowing	4,6,8.	Present study
	Intermediate centripetal furrowing	1,7,10.	
	Successive centripetal furrowing	2,3,4.	
	Successive centrifugal cell plate formation	12	
Morphology of tapetum	Dimorphic	4,6,7,8	
	Dimorphic but partly trimorphic occasionally	4	
	Regularly trimorphic	1,2,3,12	
Physiology of tapetum	Secretory	1,2,6,7,8,12	
	Partly secretory and partly periplasmodial	3	
Tapetal nuclei	Bi or multinucleate	1,2,3,4,6,7,8	
	Uninucleate	12	
Pollen grains at shedding	Single grains	4,7,8	
	Dyads	6	
	Loosely held tetrads	1,2,3	
	Tetrad enclosed by common wall	12	

***1**, *Annona muricata*; **2**, *A. reticulata*; **3**, *A. squamosa*; **4**, *Artabotrys odoratissimus*; **5**, *Asimina triloba*; **6**, *Cananga odorata*; **7**, *Goniothalamus* sp. **8**, *Miliusa wightiana*; **9**, *Monodora* sp; **10**, *Saccopetalum tomentosum*; **11**, *Uvaria* sp. and **12**, *Xylopia nigricans*.

The anthers of *Xylopia nigricans* have to be interpreted as multi-sporangiate because the archesporia themselves are many, at any rate more than 4. In *Annona* and *Cananga* on the other hand the hypodermal layer differentiates as a morphologically distinct and continuous archesporium in each corner of the anther before the onset of sterilization and hence the anthers are tetrasporangiate. However, some of the archesporial cells do not give rise to sporogenous cells. Consequently the septa of *Xylopia* and those of *Annona* differ morphologically, the former arising from the undifferentiated tissue of the anther primordium and the latter from sterilized sporogenous cells. Functionally also all the septal cells of *Annona* become tapetal, whereas in *Xylopia*, only those that border on the microspore mother cells acquire this function and the rest form part of the connective. Discontinuous differentiation of the archesporium as two groups occurs rarely in *C. odorata* (Periasamy and Swamy 1959).

In the gamut of variation in the microsporangia of the Annonaceae, the polyarchesporial condition in *X. nigricans* may be considered as most primitive. However, the initial differentiation of hypodermis indicates that its polyarchesporial nature is on its way to yield place to a tetra-archesporial condition. Polyarchesporial and polysporangiate condition similar to that in *Xylopia* occurs in *Aegiceras corniculatum* (Periasamy and Sivaramakrishnan 1979).

The tapetum is trimorphic in *Xylopia* as in *Annona* but secretory and its uninucleate condition has not been reported in any other member of the Annonaceae (table 1).

Besides all the possible variations of cytokinesis of the microspore mother cell by furrowing already reported in the Annonaceae (table 1), *X. nigricans* shows the only other possible variation viz. successive division by cell plate formation. Furthermore it exhibits the unique condition of producing a single tetrad in a micro-sporangium and the tetrad being enclosed by a common wall without a perceptible individual wall for each spore.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 4, August 1988, pp. 257–259.
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Endosperm controls symmetry changes in the developing embryos of angiosperms

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Abstract. During development the angiosperm embryo passes through the filamentous, globular and the cotyledonary phases after undergoing symmetry changes at each of these three steps. Evidences are presented to implicate endosperm control on embryo symmetry.

Keywords. Endosperm; embryo symmetry.

It is a well known fact that the dicot embryo passes through 3 distinct morphological phases during its ontogeny, the filamentous, globular and cordate phases. The filamentous phase results through the variable number of transverse divisions in the zygote. The globular phase is initiated by two successive, vertical divisions at right angles to one another in one, two or rarely more of the outermost cells of the filamentous proembryo (Periasamy 1977). The globular embryo comprises a variable number of cells depending upon the plant, but in all cases it culminates in the initiation of the *Epiphysis* at the shoot pole and of the *Hypophysis* at the root pole. The differentiation of these two poles is a very important morphogenetic step because the further orderly development of the embryo depends on whether these two poles are formed or not (Swamy and Krishnamurthy 1975, 1977). The absence of the differentiation of epiphysis and hypophysis fails to induce the transition from globular to the cordate embryo. For example, if epiphysis is not differentiated the cotyledons do not develop. As an example of this we can cite the reduced mutant type of tomato, where Caruso (1968) reported that the globular embryo does not prepare itself for the transition to the heart-shaped stage and does not have a normal shoot apex (i.e. epiphysis). The epiphysis becomes the quiescent part of the shoot apical meristem while the hypophysis forms the quiescent centre of the root apical meristem. The globular embryo then switches over to the heart-shaped one through the initiation of two lateral cotyledonary primordia from the embryonal axis nearer to the epiphysis.

In the monocotyledons also the filamentous and globular stages are met with but instead of the cordate stage the embryo assumes a different contour due to the production of only one cotyledon.

All the 3 morphological phases met with during embryogeny also involve conspicuous changes in symmetry. From the filamentous phase to the globular phase the embryo becomes typically radially symmetrical, while symmetry is lost in the next phase. In dicots, radial symmetry of globular embryo is changed into bilateral symmetry due to the initiation of two cotyledons, while in monocots symmetry is unilateral due to the initiation of only one cotyledon.

Only two possible explanations can be given for the orderly development of the embryo and for the regular transitions in the symmetry of the embryo during its ontogeny. The embryo itself may exert self regulation or that the control may emanate from the surrounding endosperm. While we cannot totally disregard the first explanation, there also appears to be some evidence for the second.

Endosperm failure invariably results in the arrest of embryo development. A careful analysis of the literature reveals that there is a close correlation between the stage of endosperm development and the stage of embryo development. At the various crucial stages of embryogeny, specific physiological and morphological changes appear to take place in the endosperm also. The morphological changes are not very clearly distinguishable in the cellular endosperm but in many nuclear and helobial endosperms at least 3 morphological phases in endosperm development (although slightly overlapping) could be distinguished—free nuclear phase, cell wall formation phase and the phase in which the storage products are deposited. Corresponding to these 3 phases, the embryo also shows distinct changes in symmetry. During the free nuclear phase, the full development of the globular phase is completed; at the time of initiation of wall formation in the nuclear endosperm, the hypophysis and epiphysis are differentiated and the cotyledonary primordia are initiated in the embryo. When the storage products are deposited the rapid development of the cotyledon (s) is noticed.

The endosperm and embryo development in at least a few cases of seed failure have brought to light the importance of the stage of cell wall formation in the nuclear endosperm in bringing about the transition from globular to the heart-shaped phase of the embryo. If cell wall formation is either absent or delayed in the nuclear endosperm of these abnormal cases, there appears to be a corresponding absence or delay in the transition of the embryo from radial to bilateral (or unilateral) symmetry. As long as the endosperm does not become cellular, the globular embryo remains globular either without addition of cells or even if cells are continued to be added, the globular form is maintained. A very good instance of this is seen in *Argemone mexicana* treated with colchicine (Chopra and Rai 1958). Here the ontogeny of the embryo until the globular stage is normal but it does not attain the pre-heart shape. Instead, it continues to increase in size without any differentiation until it is almost twice the size of the control embryos; it stops to increase further and becomes arrested. The endosperm in this case never becomes cellular. In some *Citrus* crosses (Esen and Soost 1973) the triploid embryos obtained never differentiated into a plumule-radicle axis with two cotyledons, but proliferations of various sizes were produced from the globular embryos due to continued mitotic activity. Here also the endosperm, without becoming cellular, degenerated very early.

A careful perusal of the literature concerning the *in vitro* ontogeny of embryoids reveals that transitions from filamentous to globular and from globular to heart shaped stage are possible only if the constituents of the culture medium are changed quantitatively, qualitatively or both. In fact pioneering *in vitro* studies by Steward (1958) on carrot phloem cells revealed this aspect very clearly.

Till now there is not even a single embryological investigation, in which an attempt has been made to study the various embryological phenomena within the flower in a correlative manner. Endosperm development has been studied independent of embryo ontogeny and a stagewise correlation of the events in the two structures has never been attempted. The histochemical and ultrastructural changes that take place at various stages of endosperm development should be studied in collaboration with similar studies on the embryo at various stages of ontogeny. Such studies are likely to reveal that changes in endosperm constitution at crucial times bring about crucial changes in embryo symmetry.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 4, August 1988, pp. 261–268.
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Wandlabyrinthe in the embryo sac of *Nigella damascena* Linn.

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MS received 7 December 1987

Abstract. The embryo sac of *Nigella damascena*, with wall ingrowths over entire surface and distinctly formed wandlabyrinthe at the poles, displays a well integrated model for short distance transport of nutrients. The degenerating nucellus creates an influx of metabolites into the embryo sac. The embryo sac wandlabyrinthe play a dual role—initially aid with the influx of metabolites into the embryo sac, but later participate in endosperm wall formation. The two synergid apices exhibit prominent filiform apparatus which function differently. One of the synergids degenerates immediately after fertilization where filiform apparatus helps in receiving the pollen tube. The other undamaged synergid persists along with filiform apparatus upto the octant embryo stage and this filiform apparatus probably facilitates short distance metabolite transport into the synergid. The antipodal cells show 3 types of wall ingrowths— (i) wall ingrowths at the antipodal-nucellus interface which are long, branched and spread fan-like into the antipodal cytoplasm, indicating the flow of metabolites into the antipodes from the nucellus, (ii) inter-antipodal wall-ingrowths that are small, papillate and present on both sides of the wall indicating the exchange of metabolites between the antipodes and (iii) wall labyrinths at the antipode-central cell interface which are very small and are directed into the antipode cytoplasm.

Keywords. Embryo sac wall; synergids; antipodes; wall ingrowths.

1. Introduction

Since the publications of Gunning and Pate (1974) the occurrence of wandlabyrinthe (wall ingrowths) have been reported in numerous plant tissues including the cells of female gametophyte (Schulz and Jensen 1968; Marinos 1970; Rifot 1973; Zhukova and Sokolovskaya 1977; Vijayaraghavan and Bhat 1984). A correlative histochemical study of the egg apparatus, the central cell and the antipodes is, however, lacking. *Nigella damascena* proved an ideal material for such a study. The present paper describes the presence of wandlabyrinthe on the embryo sac wall and at the interfaces of both the antipodes and the synergids with the nucellus.

2. Materials and methods

Ovules of *N. damascena*, at progressive stages of development, were fixed in precooled 10% aqueous Acrolein for 24 h at 0°C. Dehydration, infiltration and embedding were carried out as per the method of Feder and O'Brien (1968). An AO Spencer Rotary microtome, fitted with a locally devised adaptor, was used to cut 2 μm thin, sections using glass knives. Periodic acid Schiff's (PAS) reagent was used for the localization of insoluble polysaccharides (Jensen 1962). A few slides were counter-stained with Aniline blue-black (Fisher 1968). Coomassie brilliant blue was also used to localise total proteins.

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*Since deceased.

3. Results

The embryo sac of *N. damascena* is 8 nucleate, lodged in an anatropous ovule and is of the Polygonum type (Maheshwari 1950). The mature embryo sac shows an egg apparatus with two synergids and an egg at the micropylar end, two polar nuclei in the central cell and 3 antipodal cells at the chalazal end. After fertilization, the embryo sac wall elongates at the chalazal pole thus causing the antipodes to shift to a lateral position (figure 1f). The synergids, the central cell and the 3 antipodal cells show well-developed wall ingrowths both before and after fertilization.

3.1 *Synergids*

The synergid apices show well developed, elaborate filiform apparatus (FA) with extensive finger-like projections. They extend upto 1/3 the length of the synergid cytoplasm. The FA stains intensely with PAS reaction (figure 1a, b) but they are, however, protein negative. The synergid cytoplasm takes dense stain for proteins and polysaccharides. After fertilization, one of the synergids, along with the FA, degenerates, whereas the other synergid remains undamaged. The FA of the undamaged synergid persists upto the octant embryo stage.

3.2 *Central cell*

The central cell wall shows over its entire inner surface, before and after fertilization, prominent, intensely PAS positive wall ingrowths (figure 2a) that are always directed into the embryo sac. These wall ingrowths, before fertilization, are small and papillate (figure 2a) but after fertilization increase in length, branch and become labyrinth of wall material (figure 1c, d). The central cell cytoplasm is seen adpressed to the antipodal cells and shows numerous polysaccharide grains. At about the late preglobular embryo stage the wall ingrowths develop uniformly over the inner boundary of the embryo sac wall, extend into the coenocytic endosperm and divide the endosperm nuclei into compartments (figure 1e). These wall ingrowths traverse the embryo sac cavity from both sides and anastomose in the centre.

3.3 *Antipodal cells*

The antipodal cells, after fertilization, occupy a lateral position due to the elongation of the embryo sac at the chalazal end (figure 1f). A few nucellar cells positioned below

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Figure 1. a, c and d. After PAS-staining. **b.** Coomassie brilliant blue stained. **e and f.** After PAS-aniline blue-black staining. **a.** Longisection of ovule showing the synergid cytoplasm and densely PAS-stained FA at the micropylar end of the embryo sac (× 185). **b.** Longisection of the synergids with FA at the micropylar end of the embryo sac. The FA do not stain with Coomassie blue (× 260). **c.** Intensely stained embryo sac wall projections (× 290). **d.** Ovule showing embryo sac wall ingrowths. The nucellus is two layered and outer integument cells show lyses (× 220). **e.** Longisection of ovule showing late proembryo and endosperm nuclei interspersed between embryo sac wall projections (× 110). **f.** The embryo sac elongates at the chalazal end and results in lateral position of antipodes. The embryo sac wall also shows fibrillar wall ingrowths (× 110).

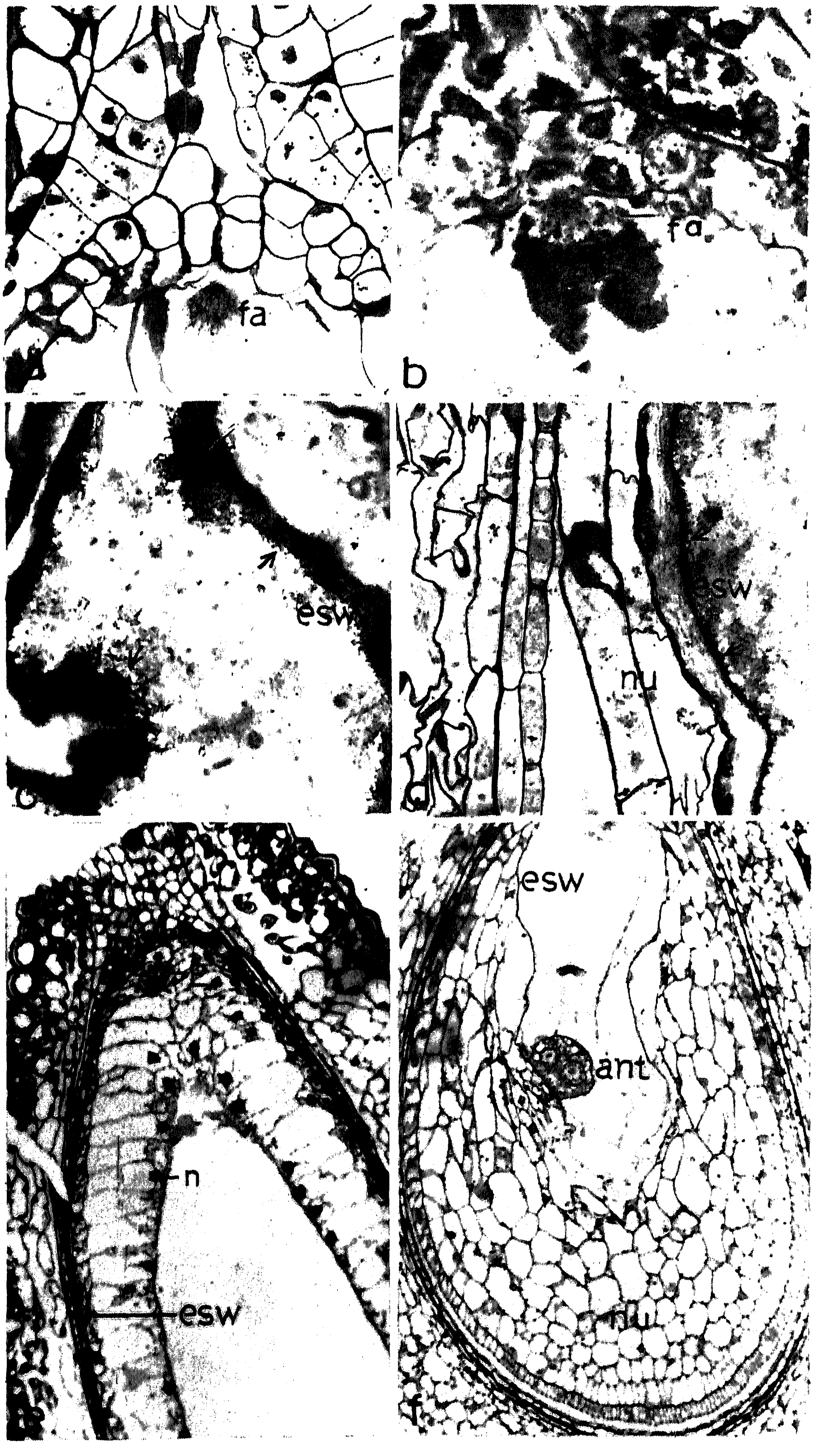

Figure 1.

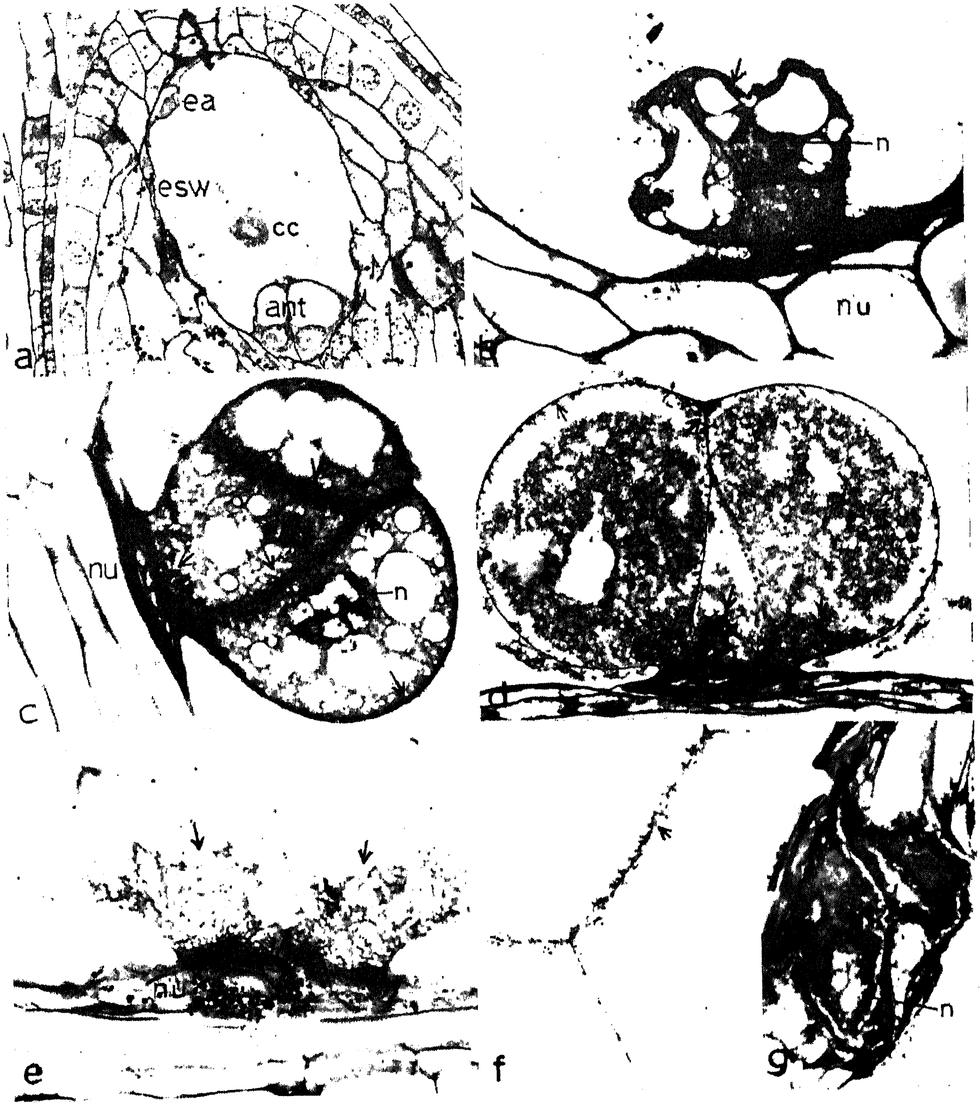

Figure 2. **a–d and g.** After PAS-aniline blue-black staining. **e and f.** After PAS staining. **a.** Ripe unfertilized embryo sac with the central cell and antipodal cells. The antipodal cells show negligible wall projections (× 115). **b.** Part of the fertilized embryo sac showing the antipodal cells with small wall projections at the base (× 260). **c.** The antipodal cells at the 2-celled embryo stage, show small wall projections at the base of the antipodal cells. The walls separating the 3 antipodal cells show small papillate wall projections. The boundary wall of the antipodal cells also shows dense wall projections (× 330). **d.** Antipodal cells at 3-celled proembryo stage, are based on a small nucellar stalk and show prominent well branched wall projections at the base. The walls separating antipodal cells show thinner wall projections (× 460). **e.** Enlarged view of the antipodal cells showing the nucellar stalk with numerous PAS grains and the antipodal cells showing the wall projections at the base (× 610). **f.** The interantipodal walls thin down considerably and show a beaded appearance (× 610). **g.** Longisection of ovule showing the degenerating antipodal cells at the late proembryo stage. The degenerating antipodal cells show few wall projections (× 280).

(ant, Antipodal cells; cc, central cell; ea, egg apparatus; esw, embryo sac wall; n, nucleus; nu, nucellus; sy, synergid; ↓ wall ingrowths).

the antipodes invariably take dense stain for proteins and polysaccharides and are compactly arranged compared to the remaining nucellar cells (figure 1f). These cells, during further progressive stages, accumulate polysaccharide grains and present a stalk-like structure over which the 3 antipodes are perched. The antipode cytoplasm takes moderate stain with PAS reaction whereas the antipode walls stain well for polysaccharides (figure 2b–d). The antipodal nuclei show large quantity of distinctly oriented heterochromatin and large nucleoli with numerous vacuoles (figure 2a–c). Immediately after fertilization the antipodal cells also display numerous vacuoles which decrease during the progressive stages and finally disappear (figure 2d). The antipodal cell walls are endowed with 3 types of wall ingrowths— (i) those at the antipodal-nucellar interface which are long, branched that spread fan-like into the antipode cytoplasm. No wall ingrowths are present towards the nucellus (figure 2b–e), (ii) inter-antipodal walls that are small and papillate and present on both sides (figure 2b, c) and (iii) antipode-central cell interface shows very small wall ingrowths that are invariably directed into the antipode cytoplasm (figure 2b, c). Before fertilization, these wall ingrowths are poorly developed (figure 2a) but after fertilization many small protuberances of PAS positive wall material are observed at the antipode-nucellar interface (figure 2b). At the zygote stage, the inter-antipodal walls reveal maximum development after which they gradually thin down. The wall ingrowths at the antipode-central cell interface also gradually thin down during the progressive stages. The antipodal cells attain a large size and continue to increase in size until about the 4 celled proembryo stage when the wall ingrowths at the antipode-nucellar interface increase in length and density and traverse to about 1/4 the length into the antipode cytoplasm (figure 2e). These wall ingrowths are very well developed, branch considerably and stain intensely with PAS reaction. The inter-antipodal walls also thin down considerably and present a beaded appearance (figure 2f). By about the late proembryo stage, the antipodal cells begin to lyse and concomitantly the wall ingrowths also breakdown (figure 2g).

4. Discussion

The highly specialised cellular adaptations in which wandlabyrinthe increase the surface area of the plasma membrane are termed 'wall membrane apparatus' and such cells with these adaptations as 'transfer cells' (Gunning and Pate 1969, 1974). In this context, the central cell, synergids and antipodal cells of *N. damascena* may be referred to as 'transfer cells'. Such cell types with wall ingrowths were also reported in *Capsella bursa-pastoris* (Schulz and Jensen 1968), *Linum usitatissimum* (Vazart 1969), *Paspalum longifolium* (Yu and Chao 1979), *Helianthus annuus* (Newcomb and Steeves 1971), *Aquilegia vulgaris* (Rifot 1971, 1973), *Conium maculatum* (Dumas 1978) and *Iberis amara* (Prabhakar and Vijayaraghavan 1983).

In *N. damascena* the wall ingrowths are well integrated into the structure of the female gametophyte with its set of wall membrane apparatus at the micropylar and chalazal ends of the embryo sac and also in the central cell. These wall ingrowths in the embryo sac cells show a temporal relationship. The development of wall ingrowths initiated at the micropylar end with the formation of FA in the synergids, proceeds toward the central cell and finally, after fertilization, culminates in the massive growth of wall projections within the antipodal cells. In *N. damascena*, except for the central cell, the maximum development of wall ingrowths is always followed by senescence, thereby implying the temporal importance for the development of wall ingrowths in the various cells of the gametophyte. Even spatially these wall ingrowths exhibit a set pattern. At the micropylar end, before and after fertilization, the FA consists of numerous wall ingrowths which are papillate and small. Similarly, the embryo sac wall ingrowths are fibrillar and short before fertilization, but after fertilization prolong excessively and participate in the endosperm compartmentation. In comparison, the wall ingrowths of the antipodal cells become massive and branched only after fertilization. Thus the set of wall membrane apparatus in the different cells of the embryo sac have strongly marked individual functions and aid in bringing about an influx of nutrients generated by the disintegrating nucellus into the embryo sac.

The FA of the two synergids in *N. damascena* function differently. While the FA of the degenerating synergid may have assisted in directing the pollen tube growth (Vijayaraghavan and Bhat 1984), the FA of the undamaged synergid persists until after fertilization and may facilitate short distance transport of nutrients into the synergids. The general absence of wall at the chalazal end of the synergids and the presence of cell wall contours at the micropylar end of synergids to form FA, endorses the role of synergids as transfer cells. The synergids due to their proximity to the egg cell may aid in nourishment.

The central cell wall ingrowths are present both before and after fertilization.in *N. damascena* as also reported in *H. annuus* (Newcomb and Steeves 1971). In *C. bursa-pastoris* they develop only after fertilization and continue to increase in size even during the heart-shaped embryo stage (Schulz and Jensen 1968, 1969). In *Lobelia*, both the chalazal and micropylar ends show wall projections during embryogenesis (Torosian 1971). In *I. amara* wall ingrowths at the chalazal end become prominent only after the heart-shaped embryo stage (Prabhakar and Vijayaraghavan 1983). In *N. damascena*, the wall ingrowths are found over the entire surface of the embryo sac wall. In *Pisum sativum* such wall projections are also found over the entire surface of the embryo sac wall but the quantum of elaboration is more near the micropylar end at which the growing embryo is located (Marinos 1970). In *H. annuus* embryo sac wall projections occur from a point approximately 1/3 the distance from the micropylar end of the synergids to roughly the level of the secondary nucleus (Newcomb and Steeves 1971). The embryo sac wall ingrowths in *N. damascena* play a dual role. In addition to absorbing the metabolites they also play an active and direct role in dividing the coenocytic endosperm into compartments. The involvement of central cell wall projections in compartmentalization of the endosperm has been suggested in *I. amara* and *Alyssum maritimum* (Prabhakar and Vijayaraghavan 1983), in *H. annuus* (Newcomb 1973), *stellaria media* (Newcomb and Fowke 1973) and *Quercus gambellii* (Singh and Mogensen 1976). Mares *et al* (1977) also reported endosperm wall formation through the centripetal growth of wall projections in the central cell (see also Vijayaraghavan and Prabhakar 1985).

Wall ingrowths are usually concentrated on those faces of the cell presumed to be most active in solute transfer (Gunning and Pate 1969). Applying this adage to the antipodal cells in *N. damascena* one can derive that the antipodal-nucellar interface is most active possessing the greatest degree of development of wall ingrowths. This is further emphasized by the distinctly developed nucellar cells at the bottom of the antipodal cells showing aggregation of proteins and polysaccharide grains. In *N. damascena* the inter-antipodal walls show well developed wall ingrowths whereas the antipode-central cell interface reveal wall ingrowths only toward the interior of the antipodal cells suggesting that the antipodal cells absorb nutrients from the nucellus and act as a sink. It is also felt that the degree of wall ingrowth development may also be connected in some way with the high degree of polyploidy of these cells. On the whole, the antipodal cells present a model where the nutrients are absorbed from all sides and thus act as reservoir of metabolites.

The antipode-nucellar interface wall ingrowths are reported in *Aquilegia vulgaris* (Rifot 1973), *Conium maculatum* (Dumas 1978), *Paspalum longifolium* (Yu and Chao 1979) and *Aconitum vulparia* (Bohdanowicz and Turala-Szybowska 1985). In *Linum usitatissimum* (Vazart 1969) peg-like ingrowths were observed at the basal region of antipodal cells. In *N. damascena* the wall-ingrowths at the antipode-nucellar interface extend deep into the cytoplasm as proliferations of wall material extending upto 1/4 the antipode length. In *Linum usitatissimum* (Vazart 1969), *Aquilegia vulgaris* (Rifot 1971, 1973) and *Aconitum napellus* (Zhukova and Sokolovskaya 1977) and *Scilla sibirica* (Bhandari and Sachdeva 1985) both the antipode-nucellar interface and the inter-antipodal walls show wall ingrowths, whereas in *Gasteria verrucosa* (Willemse and Kapil 1981) wall projections are prominent on chalazal wall and on the wall towards the central cell. This highly polarized distribution of ingrowths within the cells may be caused by a positive interaction with the neighbouring structures.

The structural features and functional potentialities described above, picture the cells in the embryo sac of *N. damascena* as a module, well equipped, to handle the transport of solutes between the symplast of the plant and its extra-cellular environment. The wall membrane apparatuses as seen in the embryo sac cells of *N. damascena* suggest that they may have developed only to cope with the nutrient flow into the gametophyte by increasing the surface area of the plasma membrane.

Acknowledgement

The award of a fellowship by the University to one of us (VS) is gratefully acknowledged.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 4, August 1988, pp. 269–276.
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Isolation of a cytokinin-binding protein from *Cucumis sativus* cotyledons

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MS received 11 February 1988; revised 10 June 1988

Abstract. A cytokinin-binding protein which exists as monomer and dimer was isolated from cucumber (*Cucumis sativus* L. var Guntur) cotyledons by affinity chromatography on AH-Sepharose-pi^{6}Ap > column. The protein bound to [^{3}H]-N^6-(Δ^2-isopentenyl) adenosine trialcohol. On Sephadex G-50 chromatography it gave 2 peaks corresponding to molecular weight 4000 and 8000 daltons. On sodium dodecyl sulphate-polyacrylamide gel electrophoresis, it gave only one band with an apparent molecular weight of 4000 daltons.

Keywords. *Cucumis sativus*; cytokinin-binding protein; N^6-(Δ^2-isopentenyl) adenosine; affinity chromatography.

1. Introduction

Binding proteins can be expected to be involved in the transport and mode of action of cytokinins. Cytokinin-binding proteins have been isolated from pea bud chromatin (Matthysse and Abrams 1970), wheat germ (Erion and Fox 1977; Polya and Davis 1978; Moore 1979), wheat embryo (Brinegar and Fox 1985), tobacco culture cells (Chen *et al* 1980; Hamaguchi *et al* 1985) and higher plant ribosomes (Berridge *et al* 1970; Fox and Erion 1975). Affinity chromatography on Sepharose-benzyladenine, AH-Sepharose-i^{6}A$^{\text{ox-red}}$ (periodate oxidised i^{6}A coupled to the amino group of AH-Sepharose followed by borohydride reduction) and AH-Sepharose-Kinetin riboside$^{\text{ox-red}}$ were used to isolate cytokinin-binding protein from tobacco leaves (Takegami and Yoshida 1975; Yoshida and Takegami 1977), tobacco culture cells (Chen *et al* 1980) and wheat germ (Moore 1979), respectively. The present communication describes the isolation of a cytokinin-binding protein from cucumber cotyledons using a new affinity column and characterization of the protein.

2. Materials and methods

2.1 *Materials*

The seeds of *Cucumis sativus* L. var Guntur were provided by Karnataka Seed Corporation, Bangalore. N^6-(Δ^2-isopentenyl) adenosine (i^{6}A), AH-Sepharose, Sephadex, ovalbumin, cytochrome c, myoglobin, insulin (β-chain), acrylamide, N, N^1-methylene bisacrylamide, ammonium persulphate, bromophenol blue, Coomassie brilliant blue, Tris and glycine were from Sigma Chemical Co., USA. [^{3}H]-i^{6}A trialcohol (Sp. act 2000 cpm/pmol) was prepared from i^{6}A by periodate oxidation and subsequent reduction with [^{3}H]-NaBH$_4$ (Randerath and Randerath 1969).

2.2 *Preparation of crude extract*

Washed cucumber seeds were germinated on moist Whatman No. 1 paper disc in sterilised petri plates at room temperature for 4 days in dark. About 20 pairs of cotyledons excised from embryo axes were transferred to each sterilized petri dish containing Whatman No. 1 filter disc moistened with sterile water and grown at 25°C with 90% humidity for 5 days under fluorescent lamp. About 8 g fresh weight of cucumber cotyledons were homogenized in a waring blender with 25 ml of 0·1 M phosphate buffer (pH 7·2) containing insoluble polyvinylpyrrolidone (0·1 g/g of cotyledons). The homogenate was squeezed through cheese cloth and centrifuged at 15,000 *g* for 20 min. The supernatant was frozen, thawed and then centrifuged at 15,000 *g* for 20 min. The resultant supernatant was used as the crude extract for affinity chromatography. All operations were done at 0–4°C.

2.3 *Preparation of AH-Sepharose-pi^{6}Ap > column*

i^{6}A was converted to the diphosphate, pi^{6}Ap (3′, 2′) by phosphorylation with cyanoethyl phosphate in the presence of 1-ethyl-3-(3-dimethyl amino propyl) carbodiimide (EDC) with the subsequent removal of the cyanoethyl group under alkaline conditions, according to the procedure of Humayun (1974). pi^{6}Ap (3′, 2′) was coupled to AH-Sepharose 4B through 5′ phosphate using EDC as coupling reagent as described by Halloran and Parker (1966). Humayun (1974) showed that on treatment with EDC, the 3′, 2′ phosphate cyclize to form 3′, 2′ cyclic phosphate (pi^{6}Ap >), leaving a 5′ phosphate for condensation to the amino groups. The amount of i^{6}A coupled to AH-Sepharose was spectroscopically determined after solubilization by NaOH and sodium borohydride treatment (Failla and Santi 1973), and found to be 2·7 μmol per ml of packed volume of AH-Sepharose.

2.4 *Affinity chromatography*

The crude extract from 8 g of cucumber cotyledons was chromatographed on AH-Sepharose-pi^{6}Ap > column equilibrated with 0·1 M sodium phosphate buffer, pH 7·2. The column was washed with the same buffer. The bound protein was eluted by two methods. In the first method 10 mM i^{6}A was the eluant. The free i^{6}A in the eluant was removed by dialysis against 0·1 M sodium phosphate buffer, pH 7·2. To remove the bound i^{6}A the dialysed protein was treated with 5% pyridine (Jayabaskaran and Jacob 1983) and dialysed against 0·1 M sodium phosphate, pH 7·2. The amount of protein was then estimated by the method of Lowry *et al* (1951). In the second method the proteins bound to the column were eluted with 0·1 N KOH instead of i^{6}A. KOH-eluted fractions were immediately neutralised with 0·1 N HCl and UV absorption at 280 nm monitored.

2.5 *Polyacrylamide gel electrophoresis*

Electrophoresis of the protein on analytical polyacrylamide disc gel electrophoresis (PAGE) was conducted using 7% gel in 0·1 M Tris-glycine buffer, pH 8·3 as described by Davis (1964). After electrophoresis, the protein on the gel was stained with Coomassie brilliant blue.

2.6 *Molecular weight determination*

The molecular weight M_r of the protein was estimated by gel filtration on Sephadex G-50 column (2 × 35 cm) with 0·1 M sodium phosphate buffer (pH 7·2) as eluant. Sodium dodecyl sulphate (SDS)-PAGE was also performed (Weber and Osborne 1969); the protein sample (100 μg) was dissolved in 0·06 M Tris-HCl buffer (pH 6·8) containing 2% SDS and 1% β-mercaptoethanol in a total volume of 0·2 ml and heated to 100°C for 2–5 min before application on 7·5% gel.

2.7 *[^{3}H]-i^{6}A-trialcohol binding*

The binding of [^{3}H]-i^{6}A-trialcohol to the protein was studied by nitrocellulose filter assay as described by Humayun and Jacob (1974) for antibody hapten systems.

3. Results

3.1 *Isolation of cytokinin-binding protein by affinity chromatography*

About 3·6 mg of protein was isolated from 8 g of cucumber cotyledons containing 160 mg of protein by affinity chromatography on AH-Sepharose pi^{6}Ap > column, using 10 mM i^{6}A for elution. After treatment with 5% pyridine and dialysis it did not bind to [^{3}H]-i^{6}A trialcohol. Dialysis done had not removed the bound i^{6}A whereas pyridine treatment resulted in loss of activity.

KOH has been used by Takegami and Yoshida (1975) for the elution of tobacco leaves cytokinin binding protein from Sepharose-benzyladenine column. In the next experiment the protein bound on the affinity column was eluted with 0·1 N KOH instead of 10 mM i^{6}A. The results are given in figure 1. The small peak which was expected to contain the cytokinin-binding protein was pooled and chromatographed

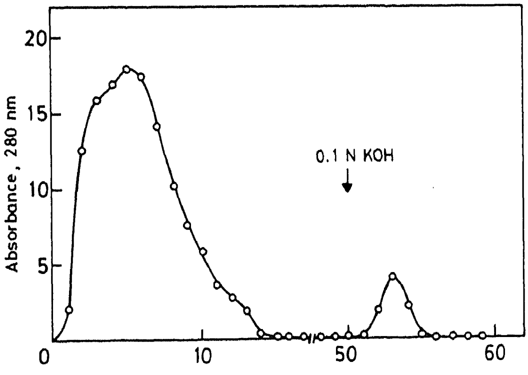

Figure 1. Isolation of cytokinin-binding protein by AH-Sepharose-pi^{6}Ap > column chromatography.

Crude extracts from cucumber cotyledons were loaded on AH-Sepharose-pi^{6}Ap > (1 × 6 cm), washed with 0·1 M sodium phosphate buffer and was eluted with 0·1 N KOH. Fractions (3 ml) were collected during loading, washing and elution.

on Sephadex G-25 column and the protein peak at the void volume was collected and used for further studies. From 8 g of cucumber cotyledons, 6·5 mg of purified protein was obtained after Sephadex chromatography.

3.2 *Binding of [^{3}H]-i^{6}A trialcohol to the KOH-eluted protein by nitrocellulose filter assay*

The optimum conditions of binding of the protein and [^{3}H]-i^{6}A trialcohol were arrived by studying the effect of pH, temperature, incubation time and protein content. The results are shown in figure 2. The binding activity is fairly constant from 4–28°C. The binding activity is very sensitive to pH changes and the maximum binding is observed between pH 7·2 and 7·5 whereas below pH 6·5 there is no binding. The maximum binding is attained in 90 min at 4°C and remain fairly constant upto 120 min. The binding activity increases linearly with the protein concentration.

3.3 *Assay for the binding of [^{3}H]-i^{6}A trialcohol by gel filtration*

The KOH-eluted protein was incubated with [^{3}H]-i^{6}A trialcohol and chromatographed on Sephadex G-25 (figure 3a). Two peaks were noticed, one at void volume due to protein-bound [^{3}H]-i^{6}A trialcohol and another due to free [^{3}H]-i^{6}A trialcohol region. On Sephadex G-50, protein-bound [^{3}H]-i^{6}A trialcohol gives two

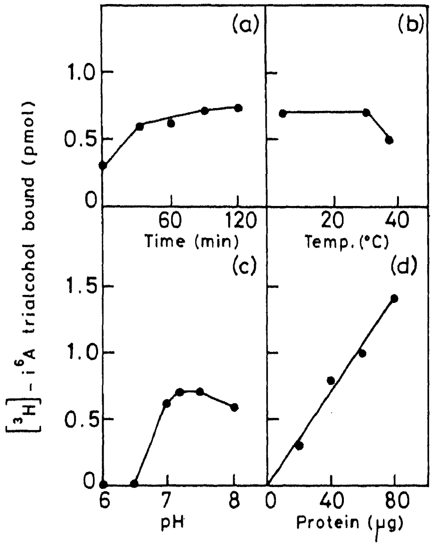

Figure 2. Effects of incubation time, temperature, pH and protein concentration on binding of [^{3}H]-i^{6}A trialcohol to the protein by nitrocellulose filter assay. **a.** Incubation time. **b.** Temperature. **c.** pH. **d.** Protein concentration.

Standard incubation procedure: Assay mixture (0·4 ml in sodium phosphate buffer, pH 7·2) containing the protein (40 µg) and [^{3}H]-i^{6}A trialcohol (10 pmol, 20,000 cpm) were incubated at 4°C for 90 min and filtered. Blank [^{3}H]-i^{6}A trialcohol only retained 383 cpm of [^{3}H]-i^{6}A trialcohol. The value plotted is after substracting the blank value.

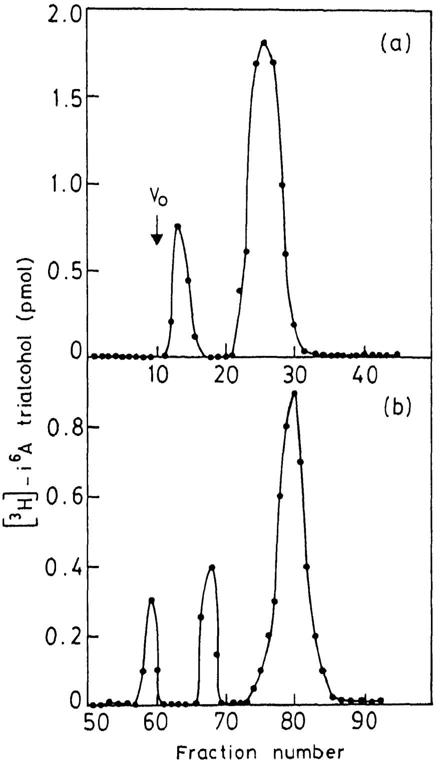

Figure 3. Binding of [^{3}H]-i^{6}A trialcohol to the protein by gel filtration. **a.** The reaction mixture (0·4 ml in 0·1 M sodium phosphate buffer, pH 7·2) containing [^{3}H]-i^{6}A trialcohol (100 pmol) and binding protein (250 μg) was incubated at 4°C for 2 h and applied onto a Sephadex G-25 column (1 × 20 cm). Fractions of 0·5 ml were collected and counted for radioactivity in a liquid scintillation counter. **b.** Assay conditions were same as in **a** except the reaction mixture was applied to a Sephadex G-50 (2 × 35 cm) column. Fractions of 1 ml were collected and counted for radioactivity.

peaks (figure 3b). The KOH-eluted protein was then chromatographed on Sephadex G-50 under the same conditions as in figure 3b. Two protein peaks were obtained. When tested for the binding of [^{3}H]-i^{6}A trialcohol by nitrocellulose assay both the peaks bound the ligand: the first peak 0·73 pmol and the second peak 0·65 pmol for 40 μg of the protein with an input of 10 pmol of [^{3}H]-i^{6}A trialcohol.

3.4 *PAGE*

PAGE of the i^{6}A or KOH eluted proteins at pH 8·3 on 7% gels shows two closely moving bands (figures 4C and D). In SDS-PAGE (7·5%) of the proteins after treatment with SDS and β-mercaptoethanol showed a single band (figure 4E). These results show that the protein exists as monomer and as aggregate.

3.5 *M_r determination*

The M_r of the monomer and aggregate as determined by Sephadex G-50 gel

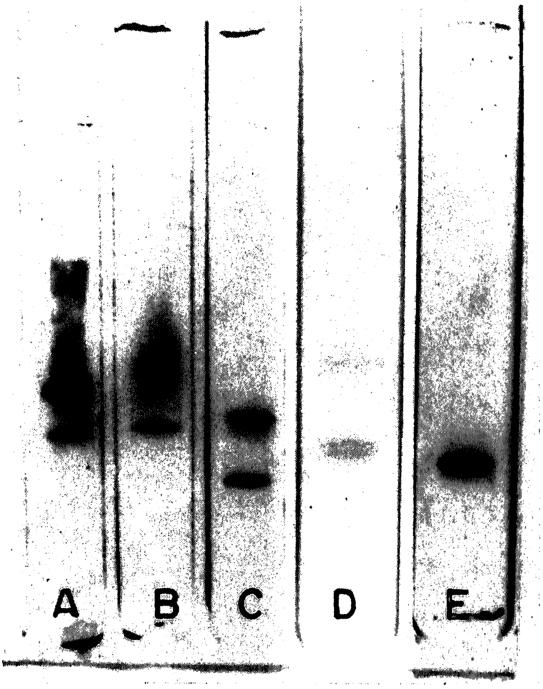

Figure 4. PAGE (7%) pattern of (**A**) crude extract (250 μg), (**B**) AH-Sepharose pi^{6}Ap > unbound proteins (130 μg), (**C**) AH-Sepharose pi^{6}Ap > 0·1 N KOH eluted protein (80 μg), (**D**) AH-Sepharose pi^{6}Ap > 10 mMi^{6}A eluted bound protein (50 μg) and (**E**) SDS-PAGE (7·5%) pattern of AH-Sepharose pi^{6}Ap > bound KOH-eluted protein (60 μg).

filtration were found to be 4000 and 8000 by reference to standard marker proteins (figure 5a). The protein on SDS-electrophoresis under reducing conditions showed a single band corresponding to the M_r of 4000 (figure 5b).

4. Discussion

By affinity chromatography on AH-Sepharose-pi^{6}Ap > and elution with i^{6}A or KOH a protein which exists as monomer and dimer (M_r 4000 and 8000) has been isolated from cucumber cotyledons. AH-Sepharose-pi^{6}Ap > column was selected for this purpose as the i^{6}A is not sterically hindered and the ribose is intact in it.

Takegami and Yoshida (1975, 1977) have reported a low M_r (4500 daltons) binding protein from tobacco leaves. Chen *et al* (1980) have reported a binding protein of M_r 8500 ± 300 daltons from tobacco culture cells. The protein that we have isolated from cucumber cotyledons also falls into the low M_r category but the gel filtration and SDS-PAGE analysis reveal that the protein exists as monomers (4000 daltons) and dimers (8000 daltons). This small M_r protein has been found to have activity both in the monomer and dimer form. High M_r cytokinin-binding proteins have been isolated from wheat germ (Erion and Fox 1977; Polya and Davis 1978; Moore 1979).

The physiological role of cytokinin-binding proteins remains to be established.

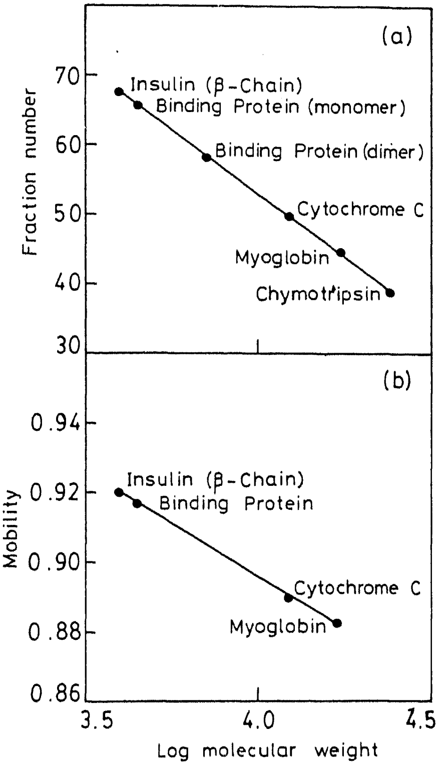

Figure 5. Determination of M_r by **(a)** Sephadex G-50 gel filtration and **(b)** SDS-gel electrophoresis.

The preliminary studies show that they may influence transcription (Matthysse and Abrams 1970). Cytokinin-binding proteins might have some role in the regulation of distribution of cytokinins within the cell or in the whole plant resulting in a characteristic response.

Acknowledgements

The author is thankful to Prof. T M Jacob for many helpful discussions, encouragement and critical reading of the manuscript. The financial assistance received from the Department of Science and Technology, New Delhi is greatfully acknowledged.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 4, August 1988, pp. 277–282.
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Growth and metabolism in cotton and tobacco callus cultures

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Abstract. Activities of key enzymes of glycolytic and pentose phosphate pathways and krebs cycle were less in tobacco callus than in cotton callus cultures grown under identical conditions. The initial period of growth was characterized by high activities of invertase, acid phosphatase, hexokinase, fructose diphosphate aldolase, malate dehydrogenase, succinate dehydrogenase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase; the activities of these enzymes declined during the remainder period of growth.

Keywords. Cotton callus; tobacco callus; growth; enzyme activities.

1. Introduction

Metabolic changes associated with wounding of plant organs have been known. However, very few details on metabolic events and the extent and timing of synthesis and activities of enzymes during growth of higher plant callus cultures are available.

Earlier studies showed that oxygen consumption increases two or more fold in the initial growth phase in callus cultures of *Acer pseudoplatanus* (Nash and Davies 1972) and potato (Akemine *et al* 1975); this was usually followed by a period of decreasing oxygen demand in both the systems. Only a few investigations have reported activity levels of enzymes during growth in cell and tissue cultures of higher plants. Such studies have shown that enzyme activities during the lag, exponential and stationary phases are varied. Our investigation provides evidence of the occurrence of some of the respiratory enzymes in cotton and tobacco callus cultures. A correlation is made between callus growth and enzyme activities.

2. Materials and methods

The initiation of cotton and tobacco callus cultures respectively from anther walls and floral buds and their maintenance have been described (Kavi Kishor and Mehta 1983, 1986). Forty ml of the medium was taken into each culture flask before autoclaving. Five replicate cultures were harvested every third day and analysed for growth and enzyme activities. Dry weight was determined by drying the tissues to a constant weight at 60°C. The calli from different cultures were pooled, weighed (fresh weight 500 mg) and homogenized at 0°C using neutral glass powder, 0·1% polyvinylpolypyrrolidone and 0·05 M phosphate buffer (pH 7). The tissue homogenates were centrifuged at 30,000 *g* for 20 min in a refrigerated centrifuge. The supernatant was collected and its volume made upto 10 ml with the buffer. The crude supernatant was used for enzyme and protein assays; table 1 lists the assay methods used. Slight modifications in pH, substrate, enzyme and co-enzyme concentrations were made as necessary in the methods referred.

Table 1. Protein and enzyme assay methods.

Substance assayed	Reference to assay method	Activity expressed* (one unit)
Amylase	Bernfeld (1955)	As 10 mg of maltose liberated/30 min
Invertase	Bacon (1955)	As quantity of enzyme required to produce 1 mg of reducing sugars/h
Acid phosphatase	Zink and Veliky (1979)	As 1 mM of *p*-nitrophenol released/min
Hexokinase (HK)	Joshi and Jagannathan (1966)	As 1 n mol of NADPH formed/min
Fructose diphosphate aldolase (FDPH)	Jagannathan *et al* (1956)	As the quantity of enzyme required to cause an increase in absorbance by a factor of 0·1/5 min
Malate dehydrogenase (MDH)	Ochoa (1955)	As 1 n mol of NADH oxidized/min
Succinate dehydrogenase (SDH)	Malik and Singh (1980)	As change in absorbance (0·1) at 600 nm/min
Glucose-6-phosphate dehydrogenase (G-6-PDH)	Filner and Klein (1968)	As 1 n mol of NADPH formed/min
6-phosphogluconate dehydrogenase (6-PGDH)	Pontremoli and Grazi (1966)	As 1 n mol of NADPH formed/min
Proteins	Lowry *et al* (1951)	—
Total sugars	Yemm and Willis (1954)	mg of glucose/100 mg of dry tissue
Reducing sugars	Nelson (1944) Somogyi (1952)	mg of glucose/100 mg of dry tissue
Total starch	Hassid and Neufeld (1964)	mg of glucose/100 mg of dry tissue

*Specific activity of enzymes was expressed as units per mg of protein.

3. Results and discussion

Both tobacco and cotton callus tissues exhibited lag and exponential phases of growth; a stationary phase was absent. The increases in cotton callus growth during 30 days on fresh weight and dry weight basis were respectively 31 and 22 fold; on the other hand, tobacco callus exhibited an increase by 34 and 41 fold respectively. In both the tissues the total sugars and the reducing sugars accumulated fast during the lag period but declined thereafter linearly. The high level of reducing sugars during the initial growth phase suggests a high invertase activity as in Paul's Scarlet Rose (Nash and Boll 1975). While starch accumulated in tobacco calli until day 9, it declined during the corresponding period in cotton calli (table 2).

All enzymes except amylase showed peak activities either in the lag phase or in the pre-exponential phase of growth in both the callus systems; during the exponential phase, the activities either remained steady or declined. Amylase showed its peak during the exponential phase in both systems. The enzyme activities were usually higher in cotton callus than in tobacco callus (table 3).

Upon transfer of cotton callus to fresh medium, considerable rise in the activity of soluble neutral invertase has been reported (Kavi Kishor and Mehta 1987). Such increases in enzyme activities have been attributed to increased protein synthesis and induction of cell division in the callus (Palmer 1968). The activity of acid phosphatase decreased sharply during the initial 3 days in both the systems, attaining its peak on day 15 in cotton and on day 6 in tobacco (table 3). The initial decline in the activity of acid phosphatase may be due to the presence of high inorganic phosphate in the medium. Studies with *Nicotiana* (de Jong 1967) and Paul's Scarlet Rose (Nash and Davies 1972) cell suspension cultures have shown a rapid rise in total malate dehydrogenase (MDH) activity until the mid-exponential

Table 2. Quantitative data on growth of cotton and tobacco callus cultures on Murashige and Skoog's medium + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l Kn and 2% sucrose.

Culture age (days)	Fresh weight (mg/culture)		Dry weight (mg/culture)		Total sugars (mg %)		Reducing sugars (mg %)		Total starch (mg %)	
	Cotton	Tobacco	Cotton	Tobacco	Cotton	Tobacco	Cotton	Tobacco	Cotton	Tobacco
0	400 (±40)	300 (±30)	16 (±4)	12 (±3)	7	3	3	3	13	9
3	503 (±43)	576 (±23)	21 (±4)	43 (±6)	30	18	14	15	13	10
6	648 (±56)	735 (±39)	27 (±4)	45 (±4)	36	15	33	13	11	11
9	1450 (±99)	2411 (±48)	54 (±6)	99 (±7)	17	12	6	9	9	13
12	2452 (±107)	3528 (±99)	97 (±7)	133 (±9)	15	10	6	9	12	11
15	3463 (±122)	4195 (±120)	127 (±9)	174 (±10)	11	9	6	5	14	11
18	5673 (±200)	4516 (±128)	191 (±11)	208 (±10)	9	7	4	5	13	13
21	6921 (±300)	6268 (±214)	220 (±13)	299 (±12)	6	6	3	4	14	10
30	12500 (±481)	10129 (±444)	357 (±20)	486 (±16)	7	3	3	3	13	9

Numbers in parentheses represent SE.
Inoculum wt for cotton 400 ± 40 mg and for tobacco 300 ± 40 mg; data represent average of 5 replicates.

Table 3. Enzyme activities (units/mg protein) during growth in callus cultures of cotton and tobacco.

Enzyme and callus system	Culture period (days)								
	0	3	6	9	12	15	18	21	30
Amylase									
Cotton	2·2	1·0	1·8	2·4	2·2	2·6	2·8	2·7	2·2
Tobacco	0·3	0·2	0·1	0·3	0·2	0·1	0·1	0·2	0·3
Invertase									
Cotton	0·3	2·1	1·6	0·7	0·8	1·5	1·0	1·4	0·3
Tobacco	0·4	0·2	0·3	0·4	0·6	0·1	0·5	0·1	0·4
Acid phosphatase									
Cotton	0·9	0·4	1·9	2·2	2·6	3·8	1·6	0·8	0·9
Tobacco	0·8	0·5	1·3	0·9	0·9	0·5	0·4	0·6	0·8
HK									
Cotton	0·7	4·9	4·3	4·6	3·5	2·3	1·0	1·2	0·7
Tobacco	0·4	1·7	1·2	1·0	0·9	0·6	0·5	0·4	0·4
FDPA									
Cotton	6·7	17·6	16·7	17·1	10·7	11·9	8·2	8·9	6·7
Tobacco	1·7	5·3	4·4	13·0	10·9	5·2	7·5	9·8	1·7
MDH									
Cotton	4·8	10·2	8·6	9·5	10·0	12·1	20·0	11·1	4·8
Tobacco	0·9	1·9	3·4	3·0	2·9	1·7	2·0	2·4	0·9
SDH									
Cotton	1·0	7·5	7·5	5·2	5·0	4·0	3·3	1·5	1·0
Tobacco	0·7	4·5	4·1	3·3	2·2	2·5	1·5	1·1	0·7
G-6-PDH									
Cotton	2·4	9·7	2·2	3·2	3·2	3·1	3·3	8·2	2·4
Tobacco	1·2	2·0	2·7	5·2	2·8	1·1	1·6	2·1	1·2
6-PGDH									
Cotton	1·6	13·3	10·3	8·0	5·8	3·0	2·2	2·7	1·6
Tobacco	0·9	6·5	6·8	4·2	3·1	2·2	2·2	1·4	0·9

Inoculum wt: cotton 400 ± 40 mg, tobacco 300 ± 30 mg. Data are average of 5 replicates.

phase of growth followed by a very rapid decline during the remainder of the exponential phase. Givan and Collin (1967) have demonstrated high respiratory rate during the lag phase of growth in *Acer pseudoplatanus* cell suspensions. The respiratory rate declined with the progress of growth. Thus, the energy required for exponential growth is produced prior to its onset. In *A. pseudoplatanus*, the lowest activities throughout the growth cycle were found in phosphofructokinase for the Embeden–Mayerhoff-Parnas (EMS) pathway, and in glucose-6-phosphate dehydrogenase (G-6-PDH) and transketolase for the pentose phosphate (PP) pathway (Fowler 1971). These enzymes were, therefore, considered rate-limiting. In our study G-6-PDH and 6-phosphogluconate dehydrogenase (6-PGDH) for PP pathway, and hexokinase (HK) and fructose diphosphate aldolase (FDPA) for EMP pathway do not seem to be rate-limiting enzymes. However, these enzymes have activity peaks that coincide with those for oxygen consumption as shown by Komamine and Shimizu (1975) in carrot callus cultures. That 6 of the enzymes showed peak activity during the lag and pre-exponential phases of growth (table 3) support the work of Fowler (1971) and Wilson (1971) who suggested that lag phase is characterized by intense metabolic activity and that the balance of metabolism is directed towards biosynthesis which required the development of reducing power generated through the PP pathway. Shimizu *et al* (1977) have reported that in sycamore cell suspension cultures the respiratory activity per cell was highest during intensive metabolic activity. During this lag phase they found high levels of ATP, NAD and NADPH, and also demonstrated the peak in energy charge of the culture. Once cell division is commenced there is a gradual change in the pattern of metabolism. However, it is important to note that the metabolic activities measured in asynchronous systems would merely represent an average value of that parameter in cells at various stages of cell cycle (Komamine *et al* 1978).

Acknowledgement

One of the authors (PBK) is grateful to the University Grants Commission, New Delhi, for the award of a fellowship.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 4, August 1988, pp. 283–290.
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Response of pigeon pea to variable levels of manganese

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MS received 14 September 1987; revised 31 May 1988

Abstract. Pigeon pea (*Cajanus cajan* (L.) Millsp.) cv. T21 was grown in refined sand at manganese levels ranging from 0·0011 to 5·5 mg l^{-1}. At less than 0·055 mg Mn l^{-1}, growth was depressed. In manganese deficient plants, there was reduction in the area and number of leaves. A reduction in seed yield was observed at low and excess manganese and was optimum at 0·55 mg Mn l^{-1}. Manganese deficient plants showed enhanced levels of peroxidase and ribonuclease activity and caused reduction in chlorophyll content, sugars, Hill activity and activities of catalase, acid phosphatase and aldolase. When manganese supply was low, manganese concentration in leaves decreased and iron, zinc, copper and phosphorus concentrations increased. Values for deficiency, threshold of deficiency and toxicity of manganese in leaves were respectively 25, 39 and 190·5 $\mu g\ g^{-1}$ and in seeds they were 10·6, 12·8 and 18·2 $\mu g\ g^{-1}$ dry matter.

Keywords. *Cajanus cajan*; enzymes; leaf tissue; manganese deficiency.

1. Introduction

Deficiency of manganese is known to affect adversely the biomass, net photosynthesis, soluble carbohydrate and chlorophyll concentrations in leaves of higher plants (Marschner 1986).

Participation of manganese in the Hill reaction has also been established (Cheniae and Martin 1968). The role of manganese either as an activator or a constituent of enzymes is well documented (Marschner 1986).

In several plant species in addition to the visible foliar symptoms, the deficiency of manganese is known to reduce tissue manganese with an increase in the concentration of some micronutrients (Sommers and Shive 1942; Agarwala and Sharma 1961; Coronel and Wallihan 1971; Ohki 1975).

The information on the critical deficiency levels of manganese is very limited especially in dicots (Ohki 1982). Therefore the present paper reports the influence of varied levels of manganese supply to pigeon pea grown in purified sand on growth, biomass production, seed yield, tissue mineral content and activities of few enzymes, Hill activity, concentrations of chlorophyll and sugars.

2. Materials and methods

Pigeon pea (*Cajanus cajan* (L.) Millsp. cv. T21) was grown in purified sand at graded levels of manganese—0·0011, 0·0055, 0·011, 0·055, 0·55 and 5·5 mg l^{-1}. The silica sand was purified by two successive treatments with 1% (w/v) oxalic acid in 17% (v/v) HCl in a 'Keebush' sand digester according to the method of Hewitt (1966).

The purification of macronutrients against manganese and the composition of culture solutions was according to Sharma *et al* (1986). The contribution of manganese from purified sand, culture solutions and water was not more than 0·0001 mg l^{-1}. There were two replicates in each treatment.

For dry matter and tissue analysis, plants were separated into tops and roots at day 28 and into leaves, stem and roots at day 60, washed with 0·01 N HCl, rinsed with glass distilled water and dried at 70°C for 24 h in a forced draught oven. Seed yield was obtained at day 182. Tissue manganese, iron, zinc and copper were determined by atomic absorption spectrophotometer and phosphorus was estimated colorimetrically by the method of Nicholas as described by Wallace (1951) after wet digestion of the oven dried material in a mixture of (10:1) HNO_3–$HClO_4$ acids (Piper 1942).

At day 37, crude tissue extracts of comparable middle leaves were assayed for catalase, peroxidase, acid phosphatase, ribonuclease and aldolase (Agarwala *et al* 1965, 1985).

Concentrations of chlorophyll and Hill activity (Agarwala *et al* 1965; Sharma *et al* 1986) and sugars (Nelson 1944) were estimated in middle leaves at day 36. Soluble protein concentration in the enzyme extracts was measured by the method of Lowry *et al* (1951).

The results were analysed statistically. Deficiency, threshold of deficiency and toxicity values were determined according to Agarwala and Sharma (1979).

3. Results

3.1 *Visible symptoms*

3.1a *Deficiency*: After 20 days of the sowing of seeds, the depression in growth of plants became apparent at 0·0011 and 0·0055 mg Mn l^{-1} supply. The middle leaves of these plants showed interveinal chlorotic mottling on which a few irregular necrotic spots appeared, later these spots coalesced to form large necrotic lesions. In another 40 days, at 0·0011 and 0·0055 mg Mn l^{-1} supply, the number and the area of leaves were reduced and the branches remained rudimentary. The manganese supply of less than 0·055 mg l^{-1} delayed flowering and severely restricted pod formation.

3.1b *Toxicity*: Except for suppression of growth, no visual symptoms of toxicity were observed in plants grown at excess (5·5 mg l^{-1}) manganese.

3.2 *Yield*

At 28 and 60 days after sowing, the dry matter increased with an increase in manganese supply from 0·0011 to 0·55 mg Mn l^{-1}, there being marked reduction in dry matter at less than 0·055 mg Mn l^{-1}. A slight decrease in yield was also observed at 5·5 mg Mn l^{-1}. Optimum dry matter was obtained at 0·55 mg Mn l^{-1} (table 1).

3.3 *Seed yield*

At 182 days after sowing, owing to acute deficiency of manganese (0·0011 mg Mn l^{-1}), almost 99% decrease in seed yield was obtained, whereas the reduction in seed yield was 80% at 0·0055 mg Mn l^{-1} and more than 60% at 0·011 mg Mn l^{-1}, about 40%

Table 1. Variable manganese supply and dry matter, grain yield and manganese content in pigeon pea.

Days after sowing	mg Mn l^{-1}						LSD at $P = 0{\cdot}05$
	0·0011	0·0055	0·011	0·055	0·55	5·5	
Dry matter: g per plant							
28	0·108	0·108	0·112	0·168	0·349	0·294	0·033
60	0·22	0·78	2·46	13·14	24·64	21·01	7·30
Leaf area: sq cm							
60	5·6	17·5	24·2	45·8	50·0	47·5	2·7
Seed weight: g per plant							
182	0·57	6·07	16·49	20·26	42·67	23·86	7·32
Tissue Mn: μg g^{-1} dry matter							
28	Tops						
	5·1	5·4	7·0	9·2	36·0	59·0	9·1
	Roots						
	9·0	9·4	13·4	14·0	14·2	30·6	5·4
60	Leaves						
	10·0	12·0	15·2	20·3	56·5	231·0	6·5
	Stem						
	4·8	5·6	5·8	5·8	9·4	23·9	5·0
	Roots						
	7·2	6·6	4·8	5·8	10·8	71·2	5·8
182	Grains						
	7·2	7·8	9·0	12·5	15·0	21·5	3·3

at excess (5·5 mg l^{-1}) manganese. Optimum seed yield was observed at 0·55 mg Mn l^{-1} (table 1).

3.4 *Tissue concentration*

3.4a *Manganese*: After 28 days of sowing, tissue manganese in both tops and roots increased with an increase in manganese supply from 0·0011 to 5·5 mg l^{-1} (table 1).

After 60 days of sowing, at all levels of manganese supply, manganese concentration in the leaves was more than in stem and roots. At this stage, the leaves appeared to be a better indicator of manganese status of pigeon pea plants than the other plant parts. The values for deficiency, threshold of deficiency and toxicity of manganese in leaves were 25, 39 and 190·5 μg g^{-1} respectively.

In grains, the manganese concentration increased with an increase in manganese supply from 0·0011 to 5·5 mg l^{-1}. Compared to 15 μg g^{-1} manganese in the grains with normal (0·55 mg Mn l^{-1}), it was between 7·2 to 12·5 μg g^{-1} in grains of plants raised at low manganese (0·0011 to 0·055 mg l^{-1}). The values for deficiency, threshold of deficiency and toxicity of manganese in seeds were respectively 10·6, 12·8 and 18·2 μg g^{-1} dry matter (figure 1).

3.4b *Iron*: At both 28 and 60 days after sowing, there was marked accumulation of iron in tops and leaves of manganese deficient (0·0011 to 0·055 mg l^{-1}) plants, which was more at day 60 than at day 28. A slight increase in leaf iron occurred at excess (5·5 mg l^{-1}) manganese (table 2).

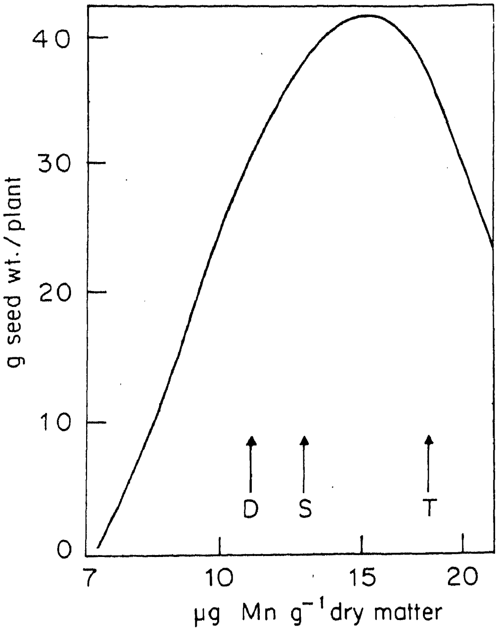

Figure 1. Values of deficiency (D), threshold of deficiency (S) and toxicity (T) of manganese in pigeon pea.

Table 2. Variable manganese supply and iron, zinc, copper and phosphorus content in pigeon pea.

Days after sowing	mg Mn l^{-1}						LSD at $P=0{\cdot}05$
	0·0011	0·0055	0·011	0·055	0·55	5·5	
Tissue Fe: $\mu g\,g^{-1}$ dry matter							
28	Tops						
	162	154	160	146	83	111	22
60	Leaves						
	584	226	226	198	111	131	75
Tissue Zn: $\mu g\,g^{-1}$ dry matter							
28	Tops						
	45·5	54·0	50·0	40·0	23·0	26·3	15·4
60	Leaves						
	81·0	34·9	34·0	17·7	16·0	20·5	13·5
Tissue Cu: $\mu g\,g^{-1}$ dry matter							
28	Tops						
	18·3	18·2	18·0	13·4	10·7	20·8	6·3
60	Leaves						
	14·9	11·0	10·0	8·6	8·7	8·0	3·6
Tissue P: % dry matter							
28	Tops						
	0·37	0·39	0·32	0·32	0·23	0·30	0·11
60	Leaves						
	0·79	0·68	0·58	0·44	0·42	0·48	0·16

3.4c *Zinc*: Compared to the values obtained at normal (0·55 mg l^{-1}) manganese level, at low manganese, the leaf zinc content increased more at day 60 than at day 28. There was slight increase at excess level of manganese also (table 2).

3.4d *Copper*: Tissue copper increased more than 1½ times at low manganese level at both day 28 and 60. At day 28, at excess level of manganese (5·5 mg Mn l^{-1}), leaf copper content was about twice as much as at 0·55 mg Mn l^{-1} and there was negligible difference in leaf copper content at the two (0·55 and 5·5 mg l^{-1}) levels of manganese supply at day 60 (table 2).

3.4e *Phosphorus*: At deficient ($<$0·055 mg l^{-1}) manganese level, the increase in phosphorus concentration in leaves was more conspicuous at day 60 than at day 28 (table 2). A slight increase in its concentration of phosphorus was noticed at high (5·5 mg l^{-1}) level of manganese also.

3.5 *Chlorophyll*

At 36 days after sowing, the concentration of chlorophyll increased with increasing level of manganese supply from 0·0011 to 0·55 mg l^{-1}. Higher level of manganese (5·5 mg l^{-1}) reduced its concentration considerably (table 3).

3.6 *Hill activity*

Owing to severe manganese deficiency, at 0·0011 and 0·0055 mg Mn l^{-1}, the Hill activity was markedly decreased and it increased with increasing supply of manganese from 0·0011 to 0·55 mg l^{-1}. Compared to that at normal level of manganese, the Hill activity was also reduced slightly at excess (5·5 mg l^{-1}) manganese (table 3) level.

3.7 *Enzyme activities*

With an increase in supply of manganese from 0·0011 to 0·55 mg l^{-1}, the specific activities of catalase, acid phosphatase and aldolase increased and those of ribonuclease and peroxidase decreased (table 3). At higher level of manganese (5·5 mg Mn l^{-1}) except for catalase, activities of all other enzymes decreased.

3.8 *Sugars*

At 0·0011 and 0·0055 mg Mn l^{-1}, the decrease in reducing sugars was more than that in non-reducing sugars. The reducing sugars showed an increase from 0·0011 to 0·55 mg Mn l^{-1}, whereas non-reducing sugars increased with an increase in manganese supply from 0·0011 to 5·5 mg l^{-1} (table 3).

4. Discussion

In pigeon pea, a deficiency of manganese produced visible symptoms on young and

Table 3. Variable manganese supply and chlorophyll, Hill activity, sugars and specific activities of catalase, peroxidase, acid phosphatase aldolase and ribonuclease in pigeon pea.

Days after sowing	mg Mn l^{-1} 0·0011	0·0055	0·011	0·055	0·55	5·5	LSD at $P=0·05$
Chlorophyll: mg g^{-1} fresh wt.							
36	0·17	0·30	0·50	0·95	1·31	1·28	0·09
Hill activity: Change in absorbance per 100 mg fresh wt.							
	0·096	0·117	0·217	0·302	0·324	0·298	0·050
Catalase: m mol H_2O_2 decomposed							
37	4·5	5·9	5·9	6·9	7·2	7·3	1·4
Peroxidase: Change in absorbance							
	0·52	0·46	0·32	0·30	0·22	0·18	0·17
Acid phosphatase: μg *Pi* liberated							
	85	84	113	143	145	130	17
Aldolase: μg triose P formed							
	283	355	356	388	400	381	76
Ribonuclease: Change in absorbance min^{-1}							
	1·98	1·50	1·04	1·04	0·85	0·81	0·51
Sugars: % fresh wt.							
36	Reducing						
	0·03	0·05	0·06	0·17	0·19	0·15	0·02
	Non-reducing						
	0·12	0·19	0·21	0·32	0·32	0·39	0·04

middle leaves resembling those described earlier for some legumes grown in culture solution or in the field trials (Cooper and Girton 1963; Weiland *et al* 1975; Hewitt 1983). As reported earlier for wheat, soybean and in other higher plants (Homann 1967; Weiland *et al* 1975; Ohki 1985), the decrease in chlorophyll concentration of pigeon pea leaves when manganese was in low supply might suggest an involvement of manganese in photosystem II (Marschner 1986).

The high concentrations of phosphorus, zinc and iron in manganese deficient pigeon pea is similar to those reported for rice (Coronel and Wallihan 1971), cotton (Ohki 1975) and soybean (Sommers and Shive 1942).

As observed earlier in manganese deficient soybean and wheat (Heenan and Campbell 1980; Ohki 1985), owing to its deficiency in pigeon pea the decrease in biomass along with low manganese content in leaves and the decrease in seed production would suggest an essential role of manganese for increased dry matter production and net photosynthesis in plants (Marschner 1986).

In manganese deficient plants, both reducing and non-reducing sugars decreased, an observation similar to that obtained in tomato and bean (Miller 1933; Vielemeyer *et al* 1969) where a decline in soluble carbohydrate level under similar conditions was found as a result of decreased photosynthesis suggesting the role of manganese in this process (Marschner 1986; Ohki 1985).

Manganese deficiency caused an increase in peroxidase activity in leaves of pigeon pea, which can be used as a means of distinguishing between iron and manganese deficiency (Bar-Akiva 1961). In contrast to the unaltered catalase activity in manganese deficient citrus plants (Vielemeyer *et al* 1969), there was considerable decrease in its activity at low manganese.

As observed earlier in radish (Agarwala *et al* 1976) and in pea (Vlasyuk and Koval'Chuk 1971), the activity of aldolase decreased and that of ribonuclease increased in pigeon pea leaves when manganese was supplied at low levels.

Low manganese supply resulted in a marked reduction in acid phosphatase activity in pigeon pea as has been reported earlier for sweet potato (Uehara *et al* 1974) indicating clearly the specific requirement of manganese for the normal activity.

Marked low Hill activity in manganese deficient pigeon pea leaves reveal the specific requirement of manganese for the water splitting and O_2-evolving system in photosynthesis (Cheniae and Martin 1968), where it has been observed that when manganese is in low supply, the first step of the electron transport chain of the light reaction is impaired and hence the determination of the photosynthetic O_2 evolution in leaves which depends on manganese supply can be a sensitive and suitable test method for characterisation of the manganese nutritional status of plants (Nable *et al* 1984).

Apart from having low manganese content in leaves, pigeon pea showed an accumulation of leaf copper in manganese deficiency, which is in consonance with the reports in barley (Agarwala and Sharma 1961).

Unlike some reports of the visible toxicity symptoms in several plants (Hewitt 1983), in pigeon pea no specific symptoms of toxicity could be observed at high manganese (5·5 mg Mn l^{-1}). The critical toxicity values of manganese in pigeon pea cv. T21 was lower than the values observed in some cultivars of pigeon pea (Edwards and Asher 1982) earlier.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 4, August 1988, pp. 291–297.
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Aflatoxin-producing fungi in stored paddy

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MS received 25 October 1986; revised 17 May 1988

Abstract. Stored paddy (150 samples) collected from 3 different storehouses in Tamil Nadu and threshed rice grains (20 samples) from local warehouses and retailers were assayed for the extent of fungal contamination by blotter and dilution plate methods. The contaminated samples that fluoresced in UV light and cultures of fungi isolated from stored samples were subjected to chemical extraction for aflatoxins. Aflatoxin B_1 was detected in 7 out of 17 isolates of *Aspergillus flavus* and 1 out of 6 *Aspergillus parasiticus*. Moisture content of the samples was correlated with the number of fungi isolated and the type of storehouse from which the samples were collected.

Keywords. Paddy; fungi; aflatoxin.

1. Introduction

Fungi are ubiquitous in stored products and have been widely studied. They can cause losses during storage, discolouration and impairment of taste of foods and feeds (Hesseltine 1974; Sreenivasamurthy 1977; Niles 1978), as well as presenting serious health hazards to livestock and man through allergies and mycotoxins (Bhat *et al* 1978). Toxin production by storage fungi in cereals, pulses and other types of foods and the toxicoses caused by them have frequently been recorded. However, there is little information on fungi and mycotoxins in paddy, the staple food of south India (Hesseltine 1976; Bhat *et al* 1978). The present communication deals with the evaluation of fungal contamination of paddy, stored in 3 different types of storehouses during post-harvest period and the efficacy of those fungi to produce aflatoxins *in vitro*.

2. Materials and methods

2.1 *Collection of samples*

After a preliminary survey of the cultivation and storage practices followed in different areas of Tamil Nadu in collaboration with the Save Grain Campaign (SGC), Madras, 150 paddy samples were collected from two villages in Tiruchi and Thanjavur districts during 1980–1982, where continuous cultivation of the short term crop (Kuruvai—110 days' duration being harvested in January, April and September) was being followed. Representative samples of stored paddy (Approx. 250 g) were collected in sterile polythene bags and were transported to the laboratory for fungal assay. The main types of storehouses in practice were (i) conventional mudbins, (ii) open room and (iii) metal bins (specially designed by the SGC). A further 20 samples of threshed rice grains were collected from the local warehouses and retailers.

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2.2 *Assay of fungal flora*

Fungal flora contaminating the stored crop was assessed in 3 ways, viz., dilution plating, blotter test as per the recommendations of the International Seed Testing Association (1966) and direct plating on Czapek Dox agar. Number of fungi was expressed quantitatively as the total count (10^3/g per sample) by dilution plating (1 ml of 10^{-3} dilution plated/petridish), and qualitatively as the number of fungal species isolated from 50 paddy/rice grains plated on the blotting paper or directly on agar. The fungi were isolated and maintained on Czapek Dox and Sabouraud glucose agar slopes.

2.3 *Assay of aflatoxins*

2.3a *Toxins in the contaminated samples*: The extent of contamination of the stored samples and the presence/absence of aflatoxins was qualitatively assayed by viewing them directly under UV light and looking for the characteristic bright greenish-yellow (BGY) fluorescence as described by Fennell *et al* (1973).

2.3b *Growth media*: The fungi isolated were screened for the toxin production in autoclaved maize medium and Richard's semi-synthetic liquid medium. Three replicates of either of the culture medium were seeded with 0·5 ml of the fungal spore suspension containing 10^5 spores/ml and were incubated for two weeks at room temperature. The culture flasks, after incubation, were steamed for 30 min. Pooled maize media and the liquid media were assayed separately for toxin content.

2.3c *Extraction of assay of toxins*: The pooled media were chemically extracted by Pons and CB (contamination brach) methods (Pons and Goldblatt 1965; Scott *et al* 1970; Jones 1972) and aflatoxins were detected by thin-layer chromatography using chloroform : methanol (97 : 3) as solvent. Samples exhibiting BGY fluorescence under UV light were also extracted and analysed for toxins as above. After chromatography, the plates were viewed under UV light for blue fluorescence of AFB_1 and AFB_2. The R_f values of the extracts were compared with that of the standard aflatoxins (B_1, B_2, G_1 and G_2) purchased from Sigma Chemical Co., St. Louis, Missouri, USA. Aflatoxin B_1 (AFB_1) was confirmed by using trifluoracetic acid with the development of the plate in chloroform : methanol (85 : 15). The plates were read in UV light and the R_f values of the treated toxins were compared with the standards (Shanta *et al* 1974; Stack and Pohland 1975). Toxin content of the sample and that produced by fungi was calculated according to the standard method and expressed as μg/g of the sample.

3. Results

The results are summarized in tables 1–3.

3.1 *Mycoflora of the stored grains*

Table 1 represents a quantitative analysis and the fungal species isolated from the stored grains in 3 storage structures and also from the rice samples collected from warehouses. The total count of fungi in mudbin samples were higher than the other two types, as estimated by dilution plating (table 1). Species of *Aspergillus* accounted for more than 40% of the total count, followed by *Penicillium* spp. (24%) and *Fusarium* spp. (13%). Incidence of other fungi accounted for only 23% of the total fungal population. Data on the percentage count as well as the percentage incidence of the fungi in dilution plating revealed the predominance of Aspergilli over other fungi (table 1). Qualitative analysis of the flora by blotter and direct plating methods yielded Aspergilli (*A. niger*, *A. flavus* and *A. fumigatus*) as the most common fungi, while members of Mucorales and *Fusarium* spp. ranked second in the order of incidence. Other fungal species were significantly low in occurrence.

3.2 *Storehouses versus fungal population*

Data summarized in table 2 relates to the number of fungal species and their percentage incidence in samples from different types of storehouses. Most fungal species (35) were isolated from the samples collected from mudbins, thus accounting for 76·1% of the total fungal species isolated, whereas the minimum number was recorded from the metalbin samples. The moisture content of the samples, as estimated by OSAW moisture meter was mudbins, 25–35%; warehouse, 20–26·5%; open room, 18–22% and metal bins, 9·5–12·5%.

3.3 *Detection and assay of aflatoxins*

Table 3 shows the extent of toxin contamination in stored paddy and rice samples. Out of 170 samples analysed, 30 samples contained *A. flavus* and/or *A. parasiticus* and yielded BGY fluorescence under UV light. When assayed for toxins by chemical extraction, 12 samples exhibiting the characteristic fluorescence (40%) were positive for aflatoxin B_1 (table 3). Of the 17 *A. flavus* isolates tested, 35·5% produced aflatoxin, whereas of the 6 *A. parasiticus* tested, only ·16·25% produced aflatoxin. Toxin contents of the samples showing BGY fluorescence as well as the *in vitro* cultures ranged between 0·045 and 0·320 μg/g of the sample. There was no marked influence or difference between the two extraction procedures employed in this study.

4. Discussion

Decomposition of organic matter by microbes and the association between majority of saprophytic and pathogenic fungi and the crops, specially during the post harvest/storage periods have been well-documented (Hesseltine 1974, 1976; Jhala 1976; Niles

Table 1. Total counts (10^3/g sample), percentage counts and frequency of occurrence of fungal genera and species on Czapek Dox agar as estimated by dilution plating.

Genus and species	Mudbins			Open room			Metal bins			Stored rice		
	Total Count	Count (%)	Frequency (%)	Total Count	Count (%)	Frequency (%)	Total Count	Count (%)	Frequency (%)	Total Count	Count (%)	Frequency (%)
Rhizopus nigricans	1·07	3·29	66·6	1·10	4·18	54·66	0·14	0·94	38·66	2·06	6·46	25·0
Mucor racemosus	1·16	3·56	77·3	0·60	2·28	23·33	0·26	1·75	26·66	0·65	2·04	15·0
M. heimalis	0·50	1·53	28·0	0·42	1·60	8·0	—	—	—	0·53	1·66	5·0
Cunninghamella echinulata	0·02	0·06	17·3	0·01	0·04	5·33	—	—	—	—	—	—
C. bertholletiae	0·01	0·03	6·0	—	—	—	—	—	—	0·30	0·94	10·0
Syncephalastrum racemosum	0·03	0·09	5·3	—	—	—	—	—	—	0·18	0·06	10·0
Chaetomium globosum	0·01	0·03	3·3	0·26	0·98	2·0	—	—	—	0·36	1·13	40·0
Aspergillus niger	6·28	19·28	100·0	7·04	26·79	100·0	4·38	29·40	76·0	6·81	21·34	75·0
A. fumigatus	1·22	3·75	56·0	2·96	11·26	44·66	0·74	4·97	17·33	1·60	5·01	55·0
A. flavus	3·06	9·39	84·0	2·32	8·83	51·33	2·26	15·18	19·33	3·25	10·18	85·0
A. oryzae	0·21	0·64	8·0	—	—	—	—	—	—	0·60	1·88	20·0
A. ochraceus	1·02	3·13	25·3	—	—	—	—	—	—	1·15	3·60	45·0
A. nidulans	0·48	1·47	13·3	0·48	1·83	14·66	—	—	—	—	—	—
A. tamarii	0·16	0·49	4·0	—	—	—	—	—	—	0·62	1·94	20·0
A. variecolor	0·54	1·66	7·33	—	—	—	—	—	—	—	—	—
A. terreus	1·20	3·68	20·6	—	—	—	0·83	5·57	10·66	—	—	—
A. versicolor	0·16	0·49	8·0	—	—	—	—	—	—	—	—	—
A. candidus	—	—	—	0·08	0·30	6·0	—	—	—	—	—	—
A. parasiticus	—	—	—	—	—	—	—	—	—	0·01	0·03	10·0
A. sydowii	0·03	0·09	3·33	0·02	0·08	2·66	0·04	0·27	2·66	—	—	—
Penicillium patulum	6·36	19·53	52·0	3·80	14·46	30·66	3·06	20·55	12·0	4·01	12·57	60·0
P. chrysogenum	—	—	—	1·43	5·44	12·0	—	—	—	1·65	5·17	35·0
P. citrinum	1·66	5·09	14·66	—	—	—	—	—	—	1·15	3·60	20·0
Fusarium moniliforme	2·30	7·06	44·66	3·92	14·91	19·33	2·70	18·13	7·33	4·62	14·48	30·0
F. oxysporum	1·42	4·36	17·33	—	—	—	—	—	—	—	—	—

F. roseum	0·80	2·45	8·0	—	—	—	—	—	—	0·58	1·82	25·0
Drechslera oryzae	0·60	1·84	9·33	0·28	1·06	8·0	0·06	0·40	5·33	0·02	0·06	15·0
Drechslera sp.	—	—	—	0·06	0·23	2·00	—	—	—	—	—	—
Memnoniella echinulata	0·01	0·03	2·66	0·18	0·68	5·33	—	—	—	0·16	0·51	10·0
Alternaria tenuis	0·86	2·64	12·0	0·32	1·22	7·33	0·18	1·21	4·66	0·18	0·56	10·0
Nigrospora sp.	—	—	—	0·15	0·57	5·33	—	—	—	—	—	—
Cladosporium herbarum	0·01	0·03	4·0	—	—	—	—	—	—	—	—	—
Trichoderma viride	0·16	0·49	12·0	0·06	0·23	3·3	0·01	0·06	2·0	0·03	0·09	25·0
T. pseudokoningi	0·04	0·12	2·0	—	—	—	—	—	—	0·01	0·03	5·0
Myrothecium roridum	—	—	—	—	—	—	—	—	—	0·15	0·47	10·0
Poecilomyces sp.	0·16	0·49	6·66	—	—	—	—	—	—	0·08	0·25	5·0
Phoma sp.	0·02	0·06	4·66	0·02	0·08	1·3	0·01	0·06	1·3	—	—	—
Cephalosporium sp.	—	—	—	0·01	0·04	0·66	—	—	—	0·01	0·03	10·0
Humicola grisea	0·01	0·03	4·00	0·01	0·04	2·0	0·03	0·20	0·66	0·01	0·03	15·0
Stachybotrys atra	—	—	—	0·01	0·04	0·66	—	—	—	—	—	—
Curvularia lunata	0·82	2·52	10·66	0·64	2·44	9·33	0·16	1·08	3·33	0·89	2·79	40·0
C. geniculata	0·26	0·80	6·0	—	—	—	—	—	—	—	—	—
Sporotrichum sp.	—	—	—	—	—	—	—	—	—	0·01	0·03	10·0
Geotrichum sp.	—	—	—	—	—	—	—	—	—	0·01	0·03	5·0
Phomopsis sp.	—	—	—	—	—	—	0·01	0·06	0·66	—	—	—
Sterile, white mycelium	0·01	0·03	1·33	0·10	0·38	3·3	—	—	—	—	—	—
Total count (10^3/g sample)	32·56			26·28			14·89			31·91		

Table 2. Analysis of stored paddy in relation to moisture percentage, total fungal species and their percentage incidence from the storehouses.

Type of storehouse	Moisture (%)	No. of species isolated	Incidence (%)
Mudbin	25–35	35	76·1
Open room	18–22	26	56·5
Metal bin	9·5–12·5	16	34·8
Warehouses	20–26·5	30	65·2

Table 3. Detection of fungal and toxin contamination in the samples by direct assay (UV fluorescence).

	Stored paddy			Rice grains	
	Mudbins	Open room	Metal bins	Warehouses and retailers	Total
Samples analysed	50	50	50	20	170
Samples positive for BGYF	7	2	1	20	30
Samples positive for toxin	3	0	0	9	12

1978; Bhat *et al* 1978; WHO 1979; Anonymous 1981). Storage fungi, among the 3 recognized categories of fungal succession on crops are important since they colonize the stored products and produce toxic metabolites (Christensen and Kaufmann 1965). More than 150 species of storage fungi belonging to 10–15 groups are known, with species of *Aspergillus* and *Penicillium* being most common (Hesseltine 1976). In our study of fungal contamination of paddy from 3 types of storehouses in Tamil Nadu, higher number of fungi was recorded in mudbin samples than in the open room or metal bin samples with Aspergilli as the most predominant group of fungi (table 1). Incidence of fungi in large numbers have, in general, been correlated with various factors like temperature and humidity in tropical countries. Observations on the relation between the moisture percentage and fungi have shown, that higher fungal count and fungal species in mudbin samples could be correlated, to a certain extent, with the higher moisture percentage of those samples (table 2). This is in conformity with other reports in the literature (WHO 1979).

Several studies in India have outlined the threat posed by fungi at different stages of cultivation and during storage, with special reference to the constant health hazard posed to the livestock and indirectly to man by the production of toxins in the stored food and feeds. *A. flavus* and *A. parasiticus* are heavy colonizers of the crop during storage, as well as potent toxin producers (Barnes 1970; Nagarajan and Bhat 1973; Bhat and Krishnamachari 1978; Anonymous 1981). Aflatoxins, one of the potent mycotoxins, have been detected by various methods, one among which is fluorescence as in maize and cotton seeds (Fennell *et al* 1973). Use of this method has suggested that this could effectively be employed to screen stored paddy and rice samples on a large scale.

Aflatoxins have frequently been associated with certain clinical conditions, e.g. Indian childhood cirrhosis (ICC) (Yadgiri *et al* 1970; Amla *et al* 1974; Dhatt *et al* 1982; Rajkumar *et al* 1982). Few of the other toxin activities of aflatoxins are hepatotoxicity, carcinogenicity and mutagenecity (Bhat *et al* 1978; WHO 1979). Bhat *et al* (1978) postulated that symptoms of patients with ICC, could primarily be attributed to the consumption of contaminated food. Elimination of such a fungal and toxin contamination in stored products could be avoided only with the improvement and proper monitoring of storage practices, specially during post-harvest practices, and effective physical or chemical detoxification mechanisms (Borker *et al* 1966; Shanta and Sreenivasamurthy 1975; Bhat *et al* 1978; Niles 1978), thereby reducing the risk on the human or animal lives.

Acknowledgement

Authors are grateful to the Department of Food, Ministry of Agriculture, New Delhi for the award of Research grant through a project.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 4, August 1988, pp. 299–305.
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Degradation of carbofuran and carbaryl by a suspension from a flooded soil incubated at 35°C and retreated with carbofuran

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MS received 28 September 1987; revised 30 June 1988

Abstract. An enrichment culture was prepared by repeated additions of carbofuran to a flooded alluvial soil that was incubated at 35°C. In a mineral salts medium inoculated with this enrichment culture and then incubated at 35°C, more than 90% of the ^{14}C-carbofuran and ^{14}C-carbaryl were lost within 5 and 3 days of inoculation respectively; during the same period no appreciable loss of both insecticides was noticed in the uninoculated medium. Carbofuran was degraded by the enrichment culture at a concentration as high as 1000 μg ml^{-1}. Addition of ammonium nitrogen to the medium temporarily slowed down the degradation of carbofuran by enrichment culture.

Keywords. Carbofuran; carbaryl; accelerated degradation; enrichment culture; flooded soil; temperature.

1. Introduction

Environmental fate of pesticides has been studied mostly under temperate conditions of developed countries. Only recently, a steady increase in the use of pesticides, in tropical and subtropical countries has prompted studies on their fate and behaviour in tropical and subtropical environments.

Among the carbamate insecticides, carbofuran (2,3-dihydro-2, 2-dimethyl-7-benzofuranyl N-methylcarbamate) is used widely for controlling brown planthopper (*Nilaparvata lugens* Stål) and other insect pests of rice. There are reports that carbofuran lost its efficacy after some years of its intensive use in rice fields of Philippines (IRRI 1977) and in corn fields of the USA and Canada (Fox 1983). Repeated applications of carbofuran to the same field year after year appear to aggravate the problem of its decreased efficacy in corn fields (Kaufman and Edwards 1983). Further studies in the USA and Canada showed accelerated microbial degradation of carbofuran in retreated soils (Felsot *et al* 1981; Read 1983, 1986; Kaufman and Edwards 1983; Wilde and Mize 1984; Chapman 1986; Felsot 1986). Particularly interesting was the very rapid degradation of carbofuran by a bacterium isolated from retreated soil (Karns *et al* 1986) and the enrichment culture developed in soils of retreated corn fields in the USA and Canada (Felsot *et al* 1981; Read 1983, 1986). However, repeated applications of carbofuran to a flooded tropical soil did not lead to significant enrichment of carbofuran degrading microorganisms (Rajagopal *et al* 1984b; Venkateswarlu and Sethunathan 1978; Siddaramappa *et al* 1978). In a recent study, repeated applications of carbofuran to a flooded soil that was incubated at a higher temperature of 35°C yielded an enrichment culture with an exceptional capacity to degrade all the added carbofuran (20 μg ml^{-1}) within 3 to 5 days (Ramanand *et al* 1988). The present study is concerned with the degradation of carbofuran by the same enrichment culture as influenced by its concentration and the presence of added ammonium nitrogen. The ability of this enrichment culture to degrade the related carbamate insecticide, carbaryl (1-naphthyl N-methylcarbamate) was also examined.

2. Materials and methods

2.1 *Insecticides and their metabolites*

Analytical grade carbaryl (99·5% purity) and carbofuran (99·5% purity) were obtained from Union Carbide (India) Ltd., Bombay and FMC Corporation, Middleport, New York, USA, respectively. N-methyl-^{14}C-carbaryl (specific activity, 59·5 mCi mmol^{-1}, 98% purity) was obtained from Radiochemical Centre, Amersham, England, while carbonyl-1-^{14}C-carbofuran (specific activity 13·3 mCi mmol^{-1}, 98% purity) was a gift from the FMC Corporation, USA. Technical formulations of 3-hydroxycarbofuran and 3-ketocarbofuran were obtained from FMC Corporation.

2.2 *Preparation of soil enrichment culture*

The soil enrichment culture with the exceptional capacity to mineralize carbofuran was the same as used in the earlier study (Ramanand *et al* 1988).

2.3 *Degradation of carbofuran and carbaryl by enrichment culture*

N-Carbonyl-1-^{14}C-carbofuran ($2{\cdot}2 \times 10^5$ dpm) or N-methyl-^{14}C-carbaryl (2×10^5 dpm) in acetone was added aseptically to 100 ml pre-sterilized Erlenmeyer flasks. After evaporation of acetone at room temperature, 20 ml portions of a sterile mineral salts medium ($MgSO_4{\cdot}7H_2O$, 0·2 g; K_2HPO_4 0·1 g; $FeSO_4{\cdot}7H_2O$, 0·001 g; $CaSO_4$, 0·04 g; distilled water, 1 litre; pH 6·2) were dispensed into these flasks. After equilibration for 24 h, the medium was inoculated with 0·4 ml of the enrichment culture developed at 35°C. Another set of the same medium was inoculated with 0·4 ml of the suspension from the unamended soil. Uninoculated mineral salts medium served as control. All the samples were incubated at 35 ± 1°C. At regular intervals, duplicate samples of each treatment were removed for residue extraction and analysis by liquid scintillation.

2.4 *Effect of ammonium nitrogen*

To study the influence of added ammonium nitrogen on the degradation of ^{14}C-carbofuran by soil enrichment cultures, the mineral salts medium was supplemented with 0, 0·96, 1·92 and 3·84 g l^{-1} of $(NH_4)_2HPO_4$ to provide 0, 200, 400 and 600 μg ml^{-1} of NH_4^+–N, sterilized by autoclaving and then added in 20 ml portions to pre-sterilized 100 ml Erlenmeyer flasks containing ^{14}C-carbofuran. The medium was then inoculated with 0·4 ml suspensions of soil enrichment culture. Uninoculated medium with the respective concentrations served as controls.

At regular intervals after incubation at 35 ± 1°C, duplicate samples were removed for residue extraction and analysis by liquid scintillation.

2.5 *Degradation in enrichment culture as a function of carbofuran concentration*

In another experiment, analytical grade carbofuran dissolved in acetone was added to pre-sterilized Erlenmeyer flasks at different concentrations. After evaporation of acetone at room temperature 20 ml portions of the mineral salts medium were added to each flask. The final concentration of carbofuran was 20, 50, 100, 500 and 1000 μg ml^{-1} of the medium. Uninoculated medium with the corresponding concentrations of the insecticide served as controls. At regular intervals after incubation at 35 ± 1°C, duplicate samples were removed for residue extraction and analysis by colorimetry.

2.6 *Extraction and residue analysis*

Residues in the medium (20 ml) from each flask were extracted 3 times with 30 ml portions of chloroform : diethyl ether (1 : 1) (Rajagopal *et al* 1984b). The extracts from the 3 extractions were evaporated to dryness at room temperature and the residues were redissolved in 1 or 2 ml of methanol. Residues in the methanol solution were separated by thin-layer chromatography as described earlier (Ramanand *et al* 1988). The standards were located by spraying successively with 2 N NaOH in absolute methanol and a solution of *p*-nitrobenzene diazoniumfluoroborate (5 mg dissolved in 25 ml of methanol and 25 ml of diethyl ether) (Archer 1976). Silica gel areas coinciding with the standards were scraped into tubes for colorimetric analysis of nonlabelled carbofuran or into 5 ml of scintillation cocktail to assay ^{14}C-carbofuran or ^{14}C-carbaryl.

For colorimetric analysis of nonlabelled carbofuran, the residues in silica gel were treated with 1·25 ml of 0·3% sodium nitrite solution, 1·25 ml of 0·2% sulfanilic acid in 1 N HCl and 2·5 ml of 4 N NaOH in a hot water bath (40–50°C) for 20 min. Silica gel was removed by centrifugation (2800 × g for 30 min) and supernatant was made up to 10 ml prior to colorimetric analysis at 490 nm (Venkateswarlu *et al* 1977). The recovery of carbofuran by this method was 85–90%.

In isotope studies the silica gel areas corresponding to authentic compounds were scraped into 5 ml scintillation cocktail (naphthalene, 60 g; PPO, 4 g; POPOP, 0·2 g; methanol, 100 ml; ethylene glycol, 20 ml and *p*-dioxan to make upto 1 litre). The radioactivity was assayed in a liquid scintillation counter (Model LSS-20, Electronics Corporation of India Limited, Hyderabad). All the counts were corrected for background and quenching.

3. Results and discussion

Enrichment culture obtained from a soil retreated with carbofuran at 35°C degraded both carbofuran and its related carbamate insecticide, carbaryl with almost equal ease. The residues decreased to about 5% of the original level in 5 days for carbofuran (table 1) and 3 days for carbaryl (table 2) in medium inoculated with enrichment culture from carbofuran-treated soil. The corresponding loss from the uninoculated medium and from the medium inoculated with suspension from unamended soil was around 10%. During the degradation of carbofuran, 3-ketocarbofuran and 3-hydroxycarbofuran were detected in small amounts; but no

Table 1. Degradation of carbonyl-^{14}C-carbofuran* at 35°C in a mineral salts medium inoculated with carbofuran amended soil-enrichment culture developed at 35°C.

		% of applied radioactivity recovered 20 ml^{-1} of medium					
Incubation (days)	Treatment	Aqueous phase	Solvent phase	Carbofuran	3-Hydroxy carbofuran	3-Keto carbofuran	Total recovery
0	Control	0·4	87·0	85·4	—	—	87·4
	Unamended	0·5	89·2	85·8	—	—	89·7
	Amended	0·4	85·7	84·1	—	—	86·1
2·5	Control	0·7	84·3	82·3	—	—	85·0
	Unamended	0·9	82·6	79·1	0·56	0·53	83·5
	Amended	1·6	8·7	5·3	0·90	1·21	10·3
5·0	Control	0·7	80·1	78·4	0·31	—	80·8
	Unamended	1·4	79·3	76·0	0·44	0·58	80·7
	Amended	2·1	4·2	2·2	0·56	0·70	5·3

*Carbonyl-^{14}C-carbofuran was added at $2{\cdot}2 \times 10^5$ dpm 20 ml^{-1} of medium.

Table 2. Degradation of N-methyl-^{14}C-carbaryl* at 35°C in a mineral salts medium inoculated with carbofuran amended soil-enrichment culture developed at 35°C.

		% of applied radioactivity recovered 20 ml^{-1} of medium			
Incubation (days)	Treatment	Aqueous phase	Solvent phase	Carbaryl	Total recovery
0	Control	0·6	86·5	85·0	87·1
	Unamended	0·5	87·2	86·2	87·7
	Amended	0·6	88·1	86·5	88·7
1·5	Control	0·6	79·2	77·4	79·8
	Unamended	1·2	79·7	78·0	80·9
	Amended	1·8	18·3	16·8	20·1
3	Control	0·9	74·0	72·1	74·9
	Unamended	2·1	70·5	69·4	72·6
	Amended	2·6	6·8	4·2	9·4

*N-methyl-^{14}C-carbaryl was added at $2{\cdot}0 \times 10^5$ dpm 20 ml^{-1} of medium.

metabolite was detected during the degradation of carbaryl. Overall loss of the radioactivity during the degradation of carbofuran and carbaryl by the enrichment culture, but not from uninoculated medium and from medium inoculated with suspension from unamended soil would suggest significant loss of the ^{14}C as volatiles possibly as $^{14}CO_2$. Both insecticides are chemically unstable under alkaline conditions (Rajagopal *et al* 1984a). The pH of the medium (inoculated and uninoculated) during the incubation period ranged from 6·2–6·54. Therefore, such small changes in pH were not responsible for very rapid loss of both carbofuran and carbaryl from the inoculated medium. Earlier evidence (Ramanand *et al* 1988) implicated microorganisms in the degradation of carbofuran by the soil enrichment culture.

The ability of the enrichment culture to degrade carbofuran was tested in the presence and absence of ammonium nitrogen. During degradation of carbofuran by the enrichment culture, carbofuran declined both in the presence and absence of nitrogen, but more rapidly in the absence of nitrogen (table 3). But, even in the presence of 600 μg ml^{-1} of ammonium nitrogen (highest level used) the insecticide decreased to less than 20% of the original level in 3 days. The low persistence of carbofuran in the presence of 600 μg ammonium nitrogen ml^{-1} may be also due to enrichment culture utilising carbofuran as major carbon source and to some extent added nitrogen. This would suggest that application of nitrogen fertilizers to acclimated soils with such very active enrichment cultures may slow down the degradation of carbofuran, but only temporarily. According to an earlier report (Rajagopal and Sethunathan 1984), application of nitrogen fertilizers increased the persistence of carbofuran in a flooded soil with low nitrogen content; but the soil used was not acclimated.

Table 3. Influence of NH_4^+-N on the degradation of carbonyl-^{14}C-carbofuran* in a mineral salts medium by carbofuran amended soil-enrichment culture developed and incubated at 35°C.

		% of applied radioactivity recovered 20 ml^{-1} of medium					
Incubation (days)	Treatment (μg ml^{-1})	Aqueous phase	Solvent phase	Carbofuran	3-Hydroxy carbofuran	3-Keto carbofuran	Total recovery
0	0 (UI)	0·4	88·2	86·4	—	—	88·6
	200(UI)	0·5	86·9	85·3	—	—	87·4
	400(UI)	0·4	87·8	85·9	—	—	88·2
	600(UI)	0·6	88·0	86·5	—	—	88·6
	0 (I)	0·4	88·2	86·4	—	—	88·6
	200(I)	0·5	86·9	85·3	—	—	87·4
	400(I)	0·4	87·8	85·9	—	—	88·2
	600(I)	0·6	88·0	86·5	—	—	88·6
1	0 (UI)	0·4	84·5	82·4	—	—	84·9
	200(UI)	0·5	83·1	81·5	—	—	85·6
	400(UI)	0·8	84·0	81·9	—	1·0	84·8
	600(UI)	1·2	83·5	81·5	—	0·45	84·7
	0 (I)	0·8	36·3	34·5	0·78	—	37·1
	200(I)	0·4	44·0	41·8	1·10	0·77	44·4
	400(I)	0·7	51·5	50·1	0·59	—	52·2
	600(I)	1·4	60·0	57·5	0·81	0·96	61·4
2	0 (UI)	0·8	82·1	80·6	—	—	82·9
	200(UI)	0·8	83·0	80·9	—	—	83·8
	400(UI)	0·6	80·3	78·4	—	—	80·9
	600(UI)	0·8	81·4	79·2	—	—	82·2
	0 (I)	0·7	10·0	7·9	0·79	—	10·7
	200(I)	1·2	14·5	11·1	0·64	1·30	15·7
	400(I)	1·2	22·6	18·5	0·86	1·60	23·8
	600(I)	1·8	39·5	35·2	1·20	0·90	41·3
3	0 (UI)	0·5	80·0	78·5	—	—	80·5
	200(UI)	0·8	80·9	79·6	—	—	81·7
	400(UI)	0·6	80·3	76·3	0·79	—	80·9
	600(UI)	0·8	78·5	75·3	0·81	—	79·3
	0 (I)	1·0	7·2	5·4	0·90	—	8·2
	200(I)	1·4	10·8	7·6	—	1·40	12·2
	400(I)	1·9	18·6	15·0	—	0·90	20·5
	600(I)	2·5	22·9	19·2	—	0·84	23·4

*Carbonyl-^{14}C-carbofuran was added at $2{\cdot}2 \times 10^5$ dpm 20 ml^{-1} of medium.

UI, Uninoculated; I, inoculated.

Table 4. Degradation of carbofuran as influenced by its concentration in a mineral salts medium* by carbofuran amended soil-enrichment culture developed and incubated at 35°C.

	Carbofuran concentration (μg ml^{-1})									
	20		50		100		500		1000	
Incubation (days)	UI	I	UI	I	UI	I	UI	I	UI	I
0	18	18	45	44	86	89	449	440	892	879
1	18	10	43	31	81	72	ND	ND	ND	ND
2	16	3·5	40	10	80	59	ND	ND	ND	ND
3	16	0	40	4	75	36	ND	ND	ND	ND
6	ND	ND	38	0	75	4	412	212	851	567
15	ND	ND	ND	ND	ND	ND	226	83	556	341
30	ND	ND	ND	ND	ND	ND	131	15	302	56

*The mineral salts medium was supplemented with 20, 50, 100, 500 and 1000 μg ml^{-1} of carbofuran.
UI, Uninoculated; I, inoculated; ND, not determined.

The enrichment culture could degrade carbofuran with great ease at all the selected concentrations (20–1000 μg ml^{-1}) of the insecticide (table 4). Carbofuran decreased to undetectable or negligible levels within 6 days of inoculation with the enrichment culture at insecticide concentrations of 20, 50 and 100 μg ml^{-1}. Even at 500 and 1000 μg ml^{-1} levels, the concentration decreased rapidly to less than 50 and 60% of the original level respectively within 6 days. Some loss of the insecticide was noticed also from the uninoculated medium especially at concentrations of 500 and 1000 μg ml^{-1}, possibly because of the volatilization of carbofuran at high temperature of 35°C.

Enrichment cultures developed earlier in our laboratory by repeated applications of carbofuran to a flooded soil at temperatures below 30°C could degrade about 65–75% of the added carbofuran or carbaryl in 30–40 days (Rajagopal *et al* 1984b). But, the enrichment culture developed at the soil temperature of 35°C, as used in this study, readily degraded both carbofuran and carbaryl to negligible levels even in 5 days after inoculation. The ability of this enrichment culture to tolerate and degrade high concentrations of carbofuran as a sole source of carbon and nitrogen may have practical implications in the detoxification of carbofuran and other related carbamate insecticides. Studies are underway to isolate the microorganism responsible for the degradation of carbofuran in this soil enrichment culture.

Acknowledgements

Authors are thankful to the Director, for the permission to publish this work; the Department of Environment, New Delhi, for funding this research programme; FMC Corporation, Middleport, New York, for providing carbofuran (both analytical and ^{14}C-labelled) and its metabolites; and the International Atomic Energy Agency, Vienna, Austria for the gift of ^{14}C-labelled carbaryl.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 4, August 1988, pp. 307–316.
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Biomass structure and net primary production of Siwalik chir pine (*Pinus roxburghii* Sarg.)-mixed broadleaf forest

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MS received 22 June 1987; revised 4 July 1988

Abstract. Biomass and net primary production of chir pine (*Pinus roxburghii* Sarg.)-mixed broadleaf forest is described. The total forest biomass (about 199 t $\mathrm{ha^{-1}}$) and net primary production (12·4 t $\mathrm{ha^{-1}\,yr^{-1}}$) did not reveal marked differences from similar aged central Himalayan chir pine forests. Trees accounted for most of the biomass and net primary production.

Keywords. Biomass; central Himalaya; chir pine; sub-Himalayan Siwalik range; *Quercus* spp.

1. Introduction

Between sal (*Shorea robusta* Gaertn. F.) and chir pine (*Pinus roxburghii* Sarg.) zones, around 1200 m, the forests in Kumaun Himalaya are markedly species-rich (Tewari and Singh 1985). These forests show a substantial representation of *P. roxburghii* individuals of which emerge above the layer constituted by a number of broadleaf species, including *Quercus* spp. These forests have been termed chir pine-mixed broadleaf forests (Singh and Singh 1984).

Community level phenology of the forest under study has been described by Ralhan *et al* (1985a, b). Rao (1984) has described population dynamics of trees. Detailed information on litter fall and litter decomposition are reported by Mehra (1984) and Upadhyay (1984), respectively.

The present study on plant biomass and net primary production of chir pine-mixed broadleaf forest is a part of an integrated ecological study in central Himalayan region. The primary objective of the study was to supplement information required for structure and functioning of this representative forest.

2. Materials and methods

2.1 *Site description*

The present chir pine-mixed broadleaf forest is located in Nalena range of district Naini Tal and lies (29° 19′ N lat. and 79° 28′ E long.) in sub-Himalayan Siwalik range at an elevation of 1350 m. The range is exclusively composed of sandstones, clays, silts, grits and conglomerate having the characteristics of fluviatile deposits. The Siwalik slopes are unstable with alternate hard and soft lithologies and are generally not suitable for agricultural purposes. This is partly due to their low water retention capacity and partly being near tectonically active zone, main boundary thrust (Valdiya 1980).

Soil is residual and shallow, with pH 6·5–6·7 and organic carbon content 1·6–3·5% (Khanna and Singh 1984).

Climate is subtropical monsoon type. Of the annual rainfall (2005 mm), nearly three-fourth occurs between mid-June to mid-September (average of 6 years) (figure 1). The mean daily temperature ranges from 7·9°C in January and 23·9°C in June. The year is divisible into 3 distinct seasons: (i) dry and warm, summer season (mid-March to Mid-June); (ii) wet and warm, rainy season (mid-June to mid-September); and (iii) dry and cold (with frequent frosts), winter season (October to February) (Singh and Singh 1984).

2.2 *Vegetation analysis*

Species composition of 3 storied chir pine-mixed broadleaf forest was studied in 1980, immediately after rainy season. Detailed methods of study are given in Singh and Singh (1984).

2.3 *Biomass estimation*

Trees were divided into girth at breast height (gbh) classes viz. 10–20, 20–30 cm, etc. and mean gbh (1·37 m) of each class was determined. The mean gbh value for a gbh class of a species was used in regression equation, developed earlier (Chaturvedi and Singh 1982; Rawat 1983) to get an estimate of mean biomass (by components) for that gbh class. Then this value was multiplied by the density of trees in that gbh class. Stand biomass was calculated by summing the biomass values across gbh classes. The regression equation used was in the form of:

$$\mathrm{Ln}\, Y = \mathrm{a} + \mathrm{b}\, \mathrm{Ln}\, X,$$

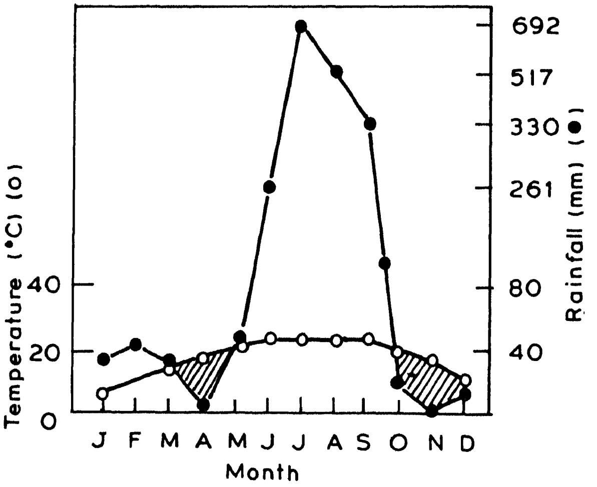

Figure 1. Climatic record for chir pine-mixed broadleaf forest.

where

> Y = dry weight of the component (kg), X = gbh (cm), a = Y intercept, and b = slope or regression coefficient.

For shrubs, 6 individuals of about the average gasal girth (girth at ground level) for different species in the forest were harvested and fresh weights of different components viz. stem (main stem + branch + twig), foliage and root were weighed separately. About 250 g subsamples for each of the components, were brought to the laboratory and ovendried at 80°C to constant weight. These values were used to convert fresh weights taken in field into dry weight. The mean biomass of individuals multiplied with density yielded total biomass for each species. The stand biomass was calculated totalling across species present.

All herbaceous plants which included seedlings of woody species, in addition to herbs were harvested at monthly interval from last week of January 1982 to last week of December, 1982, using 1 × 1 m, 10 randomly disposed quadrats. Aboveground and belowground fresh weights of components for different species were weighed in the field. Appropriate root: shoot ratio for different species was determined every month and used to avoid loss in belowground part during harvesting of the herbs. Subsamples for aboveground and belowground components were brought to the laboratory and ovendried to constant weight at 80°C. The fresh dry weight factor was used to determine the biomass. The biomass totalled across different species furnished the biomass of entire herbaceous vegetation in the forest.

2.4 *Net production estimation*

Following the establishment of a sample plot of one ha, all tree individuals of 10 cm gbh and more were marked and divided into gbh classes at 10 cm gbh intervals. The gbh was measured with a girthing tape in May 1981 and thereafter twice at an interval of one year to estimate gbh increments. For shrubs 3 individuals of about the average basal circumference for different species were marked at base (10 cm above the ground level), and increments were measured periodically as for trees. The differences between increments for two years were insignificant. The net changes in biomass ($B_2 - B_1$) yielded annual biomass accumulation. The sum of biomass accumulation in different tree components yielded net biomass accretion in the tree. Annual leaf litter fall values (Mehra 1984) were added to the foliage biomass accumulation to calculate foliage production. Wood and miscellaneous litterfall values were summed in biomass accumulation of twigs. To the fine root biomass accumulation, 1/5 of leaf litter values were added (mortality in fine roots based on the assumption of Kalela 1954; Orlov 1955, 1968; Ogino 1977) to determine fine root net production.

Shrub net production was assessed by calculating differences between biomass at time t_1 (obtained as described earlier) and time t_2 (one year interval) by allowing for the increment in basal circumference of marked individuals. To this value, foliage biomass was added by assuming 100% leaf replacement. To the root biomass accumulation, 1/5 of leaf litter was added (mortality in fine roots). The average net production multiplied by respective density yielded total net production of a species. Summing up values of all species, total shrub net production was obtained.

The annual net production for herbs was calculated by summing up all positive increments in biomass by all species (see Rana 1985).

3. Results

3.1 *Species composition*

In chir pine-mixed broadleaf forest the importance value [basal area as well as importance value index (IVI)] for trees was maximum for *P. roxburghii*, followed by *Quercus leucotrichophora* and other species (table 1). The details of shrub and herb layer analysis is given in Rana (1985).

3.2 *Biomass*

Allometric relationship between biomass of tree components and gbh are given in table 2. Tree biomass was 191·1 t ha^{-1}, of which *P. roxburghii* accounted for 42·5%; *Q. leucotrichophora* 13·1%; *Q. glauca* 5·6%; *S. ramosissima* 5·5%; *L. ovalifolia* 4·2%; and other species 29·1% (table 3). Of the total biomass in trees, maximum biomass

Table 1. Woody species composition in chir pine-mixed broadleaf forest of the central Himalaya.

Species	Total basal cover (m^2 ha^{-1})	Importance value index (IVI)
Trees		
Pinus roxburghii Sarg.	12·7	85
Quercus leucotrichophora A Camus	3·5	36
Syzygium cumini (L.) Skeel.	1·0	25
Machilus duthie King.	1·2	22
Myrica esculenta Ham. ex. D.Don.	0·8	17
Lyonia ovalifolia (Wall) Drude	0·5	17
Quercus glauca Thumb.	1·3	15
Symplocos ramosissima Wall.	0·3	14
Rhododendron arboreum Smith.	0·6	12
Pyrus pashia Buch-Ham.	0·5	9
Carpinus viminea Lindl.	0·3	9
Symplocos crataegoides Buck-Ham.	0·2	8
Engelhardtia colebrookiana Lindl.	0·1	5
Myrsine semiserrata Wall.	0·1	4
Others	0·6	14
Shrubs		
Myrsine semiserrata Wall.	0·003	73·9
Glochidion velutinum Wight.	0·003	59·8
Rubus ellipticus Smith.	0·12	38·6
Desmodium sambuense DC.	0·10	24·4
Flemingia semialata Roxb.	0·10	22·4
Debregeasia hypoleuca Wedd.	0·06	22·3
Indigofera heterantha Wall.	0·08	22·0
Rosa moschata J Harrm.	0·07	19·7
Osbeckia stellata Wall.	0·05	16·8

Table 2. Allometric relationship between the biomass of tree components (Y, kg tree^{-1}) and gbh (X cm) for different species (Ln $Y = \mathrm{a} + \mathrm{b}$ Ln X) (after Chaturvedi and Singh 1982; Rawat 1983).

Biomass (kg tree^{-1})	Intercept (a)	Slope (b)	r^2
Pinus roxburghii			
Bole	−6·418	2·598	0·985
First order branch	−9·833	2·978	0·979
Other branches	−9·338	2·630	0·963
Foliage	−6·111	1·872	0·952
Stump root	−7·220	2·448	0·974
Fine root	−9·102	2·069	0·938
Quercus leucotrichophora			
Bole	−0·523	1·367	0·994
Branch	−0·718	1·302	0·973
Twig	−0·065	0·895	0·897
Foliage	−0·976	0·854	0·299
Stump root	−0·982	0·904	0·612
Lateral root	−0·312	0·809	0·569
Fine root	−1·326	0·504	0·487
Inter species			
Bole	−0·861	1·425	0·915
Branch	−0·908	1·327	0·907
Twig	−0·506	1·028	0·796
Foliage	−1·106	1·042	0·755
Stump root	−0·098	0·948	0·789
Lateral root	−2·346	0·997	0·724
Fine root	−2·874	0·529	0·722

Table 3. Tree biomass (t ha^{-1}) in chir pine-mixed broadleaf forest.

Component	*P. roxburghii*	*Q. leucotrichophora*	*Q. glauca*	*S. ramosissima*	*L. ovalifolia*	Other species	Total
Bole	51·0	10·7	4·8	4·1	3·2	23·5	97·3
Branch	11·9	6·4	2·9	2·7	2·1	14·8	40·8
Twig	3·2	1·7	1·1	1·3	1·0	6·3	14·6
Foliage	1·7	0·6	0·4	0·4	0·3	2·1	5·5
Stump root	10·4	4·7	1·4	1·8	1·3	8·3	27·9
Lateral root	3·2	0·9	0·1	0·2	0·1	0·9	5·4
Fine root	0·2	0·1	0·01	0·02	0·01	0·1	0·4
Total biomass	81·6	25·1	10·7	10·5	8·0	56·0	191·9

was due to bole (50·7%), followed by branch (21·3%), stump root (14·5%), twig (7·6%), foliage (2·9%), lateral root (2·8%) and fine root (0·2%).

The entire shrub layer supported 5·2 t ha^{-1} biomass, of which about 75% was in the aboveground portion. Of the total biomass, ***Glochidion velutinum*** accounted for 34·6%; ***Myrsine semiserrata*** 25%; ***Rubus ellipticus*** 7·7%; and others 32·7% (table 4). The herbaceous biomass amounted to 1·7 t ha^{-1} (table 5). Of the total forest biomass (198·8 t ha^{-1}), tree, shrubs and herbs, respectively accounted for 96·5%, 2·6% and 0·9% (table 5).

Table 4. Shrub biomass (t ha^{-1}) in chir pine-mixed broadleaf forest.

Component	*G. velutinum*	*M. semiserrata*	*R. ellipticus*	Other species	Total
Stem	1·2	0·8	0·3	1·0	3·3
Foliage	0·2	0·2	0·03	0·2	0·6
Root	0·4	0·3	0·1	0·5	1·3
Total biomass	1·8	1·3	0·4	1·7	5·2

Table 5. Biomass (t ha^{-1}) of different strata in chir pine-mixed broadleaf forest.

Component	Tree	Shrub	Herb	Total
Aboveground	158·2	3·9	0·8	162·9
Belowground	33·7	1·3	0·9	35·9
Total biomass	191·9	5·2	1·7	198·8

Table 6. Tree layer net production (t ha^{-1} yr^{-1}) in chir pine-mixed broadleaf forest.

Component	*P. roxburghii*	*Q. leucotrichophora*	*Q. glauca*	*S. ramosissima*	*L. ovalifolia*	Other species	Total
Bole	1·0	0·2	0·1	0·1	0·1	0·6	2·1
Branch	0·2	0·1	0·03	0·1	0·1	0·3	0·8
Twig	0·2	0·03	0·6	0·1	0·05	0·2	1·1
Foliage	0·8	0·1	0·9	0·3	0·2	1·0	3·3
Stump root	0·3	0·4	0·01	0·04	0·03	0·1	0·8
Lateral root	0·1	0·01	0·001	0·01	0·01	0·02	0·1
Fine root	0·2	0·02	0·2	0·05	0·03	0·2	0·6
Total net production	2·8	0·8	1·8	0·6	0·4	2·4	8·8

Table 7. Shrub layer net production (t ha^{-1} yr^{-1}) in chir pine-mixed broadleaf forest.

Component	*M. semiserrata*	*G. velutinum*	*R. ellipticus*	Other species	Total
Stem	0·1	0·1	0·1	0·1	0·4
Foliage	0·3	0·2	0·04	0·2	0·7
Root	0·1	0·1	0·04	0·3	0·5
Total net production	0·5	0·4	0·1	0·6	1·6

3.3 *Net production*

The total tree net production (table 6) amounted to 8·8 t ha^{-1} yr^{-1}, of which 7·3 t ha^{-1} yr^{-1} was due to aboveground part. Of the total net production in trees, *P. roxburghii* accounted for 31·8%; *Q. leucotrichophora* 9·1%; *Q. glauca* 20·4%; *S. ramosissima* 6·8%; *L. ovalifolia* 4·5%; and others 27·3%. The apportionment of tree components was 23·9, 9·1, 12·5, 37·5, 9·1, 1·1 and 6·8% for bole, branch, twig, foliage, stump root, lateral root and fine root respectively.

Net production attributed to shrubs (table 7) amounted to 1·6 t ha^{-1} yr^{-1}, of

Table 8. Net production (t ha^{-1} yr^{-1}) in different strata of chir pine-mixed broadleaf forest.

Component	Tree	Shrub	Herb	Total
Aboveground	7·3	1·1	1·0	9·4
Belowground	1·5	0·5	1·0	3·0
Total net production	8·8	1·6	2·0	12·4

which stem, foliage and root accounted for 25, 43·7 and 31·3%, respectively. Net production of herbs was 2·0 t ha^{-1} yr^{-1} (table 8). Net production in the entire vegetation of forest (trees + shrubs + herbs) was 12·4 t ha^{-1} yr^{-1}. To this, trees shared 71%, shrubs 12·9% and herbs 16·1% (table 8).

4. Discussion

The total tree biomass (about 192 t ha^{-1}) in our study was close to that of chir pine forest (Rana 1985). Both of these forests are subjected to frequent burning and seem to have similar ages (B N Dwidi, unpublished results) and successional levels (Singh and Singh 1987). It may be pointed out that biomass values several times greater than these occur in late successional forests of the central Himalayan region (Singh and Singh 1986). This is consistent with the view that biomass increases from lower to higher level of succession (Odum 1960). Present value is also nearer to that reported for *Picea abies* forest of USSR, 197 t ha^{-1} (Parshevnikova 1957 in Rodin and Bazilevich 1967); *Abies balsamea* forest of Canada, 133–200 t ha^{-1} (Baskerville 1965); *Picea abies* forest of Belgium, 202 t ha^{-1} (Devillez *et al* 1973) and an another *P. roxburghii* forest in Kumaun region of India, 113–283 t ha^{-1} (Chaturvedi and Singh 1982; Chaturvedi 1983).

The proportion of foliage (3·5%) to the aboveground biomass in the present forest is similar to those (4·5–9·3%) reported for some conifer forests (Singh 1979, Chaturvedi 1983) and some central Himalayan oak forests (Rawat 1983; Negi *et al* 1983) and sal forests (5·1%, Singh 1979).

Tree root biomass (33·7 t ha^{-1}) in this study is comparable to that in *Picea abies* forest of USSR (33 t ha^{-1}; Remezov *et al* 1959), *Picea contorta* forest in Canada (33 t ha^{-1}; Johnstone 1971), *Pinus radiata* forest in New Zealand (33 t ha^{-1}; Will 1966), *Quercus* spp. in USSR (29–43 t ha^{-1}; Sonn 1960) and oak-pine forest in USA (36 t ha^{-1}; Whittaker and Woodwell 1969).

The shrub layer biomass in our study is higher than herb biomass. It seems that better growth of shrubs in this subtropical belt checks the herbaceous growth. Aboveground herbaceous biomass (0·8 t ha^{-1}) is lower than that of chir pine forest (1·5 t ha^{-1}; Rana 1985). Our value falls within the range of values reported (0·38–1·18 t ha^{-1}; Zavitkovski 1976) for certain temperate forests. Duvigneaud (1968) reported a total herbaceous biomass of 1·68 t ha^{-1} in an oak forest mixed with hornbeam and hazel, which is very similar to 1·72 t ha^{-1} total herbaceous biomass in the forest of present study.

Total net production under study (12·8 t ha^{-1} yr^{-1}) is close to lower values of the range reported for some *P. roxburghii* forests (9·9–21·2 t ha^{-1} yr^{-1}) of this region by Chaturvedi (1983). Our value is almost same as that reported for *Fagus sylvestris* forest in Germany (12·4 t ha^{-1} yr^{-1}; Ellenberg 1981). Whittaker and Woodwell (1971) have also reported net production value of 13 t ha^{-1} yr^{-1} for temperate forests.

Shrub net production in present study accounted for 6·2% of the total forest net production, which lies in the range (0·7–2·3 t ha^{-1} yr^{-1}) reported by Rawat (1983) for certain oak forests in Kumaun Himalaya. Aboveground herbaceous net production in our study falls in the range of values (0·1–1 t ha^{-1} yr^{-1}) reported for temperate forests (Ovington 1962). Considerably greater values than that in present forest were found for *P. roxburghii* forests of this region (1·4–2·2 t ha^{-1} yr^{-1}) by Chaturvedi (1983). Our value falls in the range reported for oak forests (1·7–2·0 t ha^{-1} yr^{-1}; Rawat 1983) of this region.

In the present study the net production was 6·7% of the total biomass. Biomass accumulation ratio (biomass/net production) has been used to characterize the production conditions in forest communities. It expresses the quantity of biomass retained per unit of net production. The ratio is largely governed by the disappearance and accumulation rate of perennial biomass. In natural forests the differences in biomass accumulation ratio result mainly due to varied condition of the size and rate of wood increment, as affected both by environmental conditions and the age of the trees. This ratio for tree layer (21·1) falls in the range of 20–50 as reported by Whittaker (1966) for intermediate and mature forests.

In central Himalaya the area under forests is far less than generally assumed, and the area under well-stocked forests is negligible (Singh and Singh 1986). Although the region has a potential to support large forest biomass (over 700 t ha^{-1}) (Rana 1985) only a small fraction of the potential biomass exists. The forests left, largely consist of early successional species and communities (i.e. chir pine forests and chir pine-mixed broadleaf forests), and oldgrowth forests are scarce. Unregulated and intense exploitation of forests and faulty management practices seem to be largely responsible for this situation. The felling cycles should have been longer than that were followed.

Acknowledgement

The authors are grateful to Prof. J S Singh, Department of Botany, Banaras Hindu University, Varanasi for constructive guideline. The financial assistance from the Department of Science and Technology and Department of Environment, New Delhi is gratefully acknowledged.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 5, October 1988, pp. 317–394.
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Contributions to our knowledge of Indian algae—3. Euglenineae Part 3. The genera *Trachelomonas* Ehrenberg and *Strombomonas* Deflandre

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Abstract. Though about 120 taxa of *Trachelomonas* and 13 taxa of *Strombomonas* are recorded from the Indian region, only about 60 taxa have been described fully. These descriptions are scattered in different journals. The present account brings together 70 taxa of *Trachelomonas* and 36 of *Strombomonas* collected from north-east, central and south India during 1937–1976, with keys and figures for their identification.

Of these, 3 species of *Trachelomonas*, viz. *Trachelomonas costatus*, *Trachelomonas assamensis* and *Trachelomonas joysagarensis* and 5 species of *Strombomonas*, viz. *Strombomonas hexagonalis*, *Strombomonas madraspatensis*, *Strombomonas vaseformis*, *Strombomonas indica* and *Strombomonas tortuosa* are considered new. Sixteen varieties and forms of *Trachelomonas* and 7 varieties and forms of *Strombomonas* are also considered new. Nine species and 12 varieties of *Trachelomonas* and 11 species and 7 varieties of *Strombomonas* are new records for the Indian region.

A list of 87 taxa of *Trachelomonas* and 7 of *Strombomonas*, the major part of which are only just listed by various authors for the Indian region, are given at the end of each genus.

Some ecological observations on *Trachelomonas* and *Strombomonas* are given. The majority of *Trachelomonas* species occurred in ponds with a fairly high degree of organic matter and fairly high nitrates and phosphates and low alkalinity during the rainless months of December to May. *Strombomonas* spp. were usually more common in ponds with higher alkalinity. *Strombomonas verrucosa* var. *conspersa* and *Trachelomonas volvocina* tolerated a wide range of total alkalinity. Excessive heat and sunlight or continuous rains seemed to have an adverse effect on the latter organism.

1. Introduction

This is the third and final part in the series 'contributions to our knowledge of Indian algae—3. Euglenineae', the first two parts having been on *Euglena*, *Lepocinclis* and *Phacus* (see Philipose 1982, 1984).

Bhatia (1930) appears to be the first to record two species of *Trachelomonas* from Kashmir. Skvortzov (1937) described 36 taxa from Rangoon. Of these, 18 taxa were considered as new. Subsequent workers have referred some of Skvortzov's taxa to other species and varieties while a few have been considered doubtful. Philipose (1940) described 12 taxa consisting of *Trachelomonas* and *Strombomonas* from Madras. Gonzalves and Joshi (1946) recorded 4 taxa from Bombay. Skuja (1949) recorded 21 taxa from Burma. Suxena (1955) described 6 taxa of *Trachelomonas* from Hyderabad. Zafar (1959) listed 4 species from the same place.

Kamat (1962, 1963) recorded 6 taxa of *Trachelomonas* from Ahmedabad, and 13 taxa of *Trachelomonas* and one of *Strombomonas* from Kolhapur, Maharashtra, respectively. Kamat (1964, 1967, 1968a,b, 1975) also reported 12 taxa of *Trachelomonas* from Bombay, two from Mount Abu (Rajasthan), 8 from Alibag (Maharashtra), 5 from Simla (Himachal Pradesh) and 45 from Vidarbha (Maharashtra) respectively. Kamat and Freitas (1976) listed 6 taxa from Nagpur.

Hortobágyi (1969) described 5 taxa of *Trachelomonas* and 3 of *Strombomonas* from 3 reservoirs on the banks of river Jamuna. Suxena *et al* (1973) reported two taxa of *Trachelomonas* and one of *Strombomonas* from Cranganore, Cochin. In 1979 Suxena described 5 species of *Trachelomonas* from high altitudes of Himalaya, and Subba Raju and Suxena (1979) described one species from Cho Oyu, Himalaya. In 1983 Suxena further described 7 species from Kodaikanal. Venkateswarlu (1976) recorded one species from river Moosi, Hyderabad. Ashtekar (1982) observed 18 taxa of *Trachelomonas* in ponds of Aurangabad, Maharashtra. Compère (1983) listed 4 species from Kashmir. Barhate and Tarar (1985) observed 4 species in Khandesh, Maharashtra.

Bongale and Bharati (1980) observed 6 *Trachelomonas* in cultivated soils of Karnataka. Hegde and Bharati (1983) listed one taxon from Bijapur while in 1986 they recorded 6 taxa forming blooms in Dharwad. Hosmani and Bharati (1983) observed 9 taxa in polluted and unpolluted waters in Karnataka. Prasad and Choudhury (1986) described a new species of *Trachelomonas* from Banaras.

Considering the overlapping in the record of many species and the fact that some taxa have been referred to other species, altogether about 55 species of *Trachelomonas* and about 64 varieties and forms have so far been recorded in the Indian region. Seven species and 6 varieties of *Strombomonas* are also known from the Indian region. With the present description of 12 additional species and 27 varieties and forms of *Trachelomonas* and 18 species and 14 varieties and forms of *Strombomonas*, there appears to be altogether 67 species and 91 varieties and forms of *Trachelomonas* and 25 species and 20 varieties and forms of *Strombomonas* for the whole of the Indian region. Out of these only about 54 taxa of *Trachelomonas* and 7 taxa of *Strombomonas* have been described fully, the rest being only listed, and these are scattered in different journals. In this paper 70 taxa of *Trachelomonas* and 36 taxa of *Strombomonas* are described with figures. A list of 87 taxa of *Trachelomonas* and 7 of *Strombomonas* described or recorded by other authors are given at the end of each genus.

2. Additional locations of collection

In addition to the locations of collection given in Philipose (1982, 1984) samples from the following places were also studied.

Assam: (1a) Sibsagar Jamuna (15–6–1955); (1b) Bighauri tank, Sibsagar (15–6–1955); (5b) NP 1, Joysagar Fish Farm (27–5–1955 and 1–6–1955); (5c) NP 4, Joysagar Fish Farm (4–6–1955); (5d) NP 16, Joysagar Fish Farm (6–4–1955 and 9–6–1955); (6a) NP 24-Joysagar Fish Farm (19–5–1955 and 30–5–1955); (19a) Namli Sarathi Pond, Nazira (15–6–1955).

Madhya Pradesh: (44a) Manzoor tank, Jabalpur (27–4–1956).

Orissa: (46a) G Mohanty's Pond, Balasore (25–11–1954); (52a) Sunda Bibi Tank, Bhadrak (28–11–1954); (56a) Shyamasunder Das Pond, Nilgiris (26–11–1954); (78a) Tank 6, Chaudwar Fish Farm (2–1–1953); (80a) NP 2, Dhenkanal Fish Farm (26–2–1957); (88a) Ramsagar Tank, Sambalpur (12–12–1954); (88b) Municipal Tank, Sambalpur (12–12–1954); (88c) Adharakonta, Sambalpur (12–12–1954); (88d) NP 7 –

Sambalpur Fish Farm (12–12–1954); (88e) NP 6–NP 6 and NP 20, Sambalpur Fish Farm (30–12–1954); (93a) NP 5–Kausalyagang Fish Farm (27–11–1952); (97a) Government Tank, Nawapara, Boudh (12–12–1954); (99a) Agra Bund, Berhampur (20–12–1954).

Andhra Pradesh: (106a) Kembala Tank, Rajamundry (6–12–1954).

Karnataka: (135a) Ramasamudram Tank, Mangalore (24–3–1974 and 28–10–1975).

Kerala: (136b) Thattapallikulam, Azhicode (10–2–1950); (136c) Palakudikulam, Azhicode (26–2–1949); (137b) Ayyappankutty Kulam, Edavankad, Azhicode (6–10–1951); (137c) Padathakulam, Azhicode (8–10–1951); (137d) Thondandikulam, Azhicode (9–2–1950); (142b) Rahinakaparamp, 2 (1–3–1949).

Tamil Nadu: (160a) Rallia Reservoir, Coonoor (12–5–1952).

3. Ecological notes

Though a wide variety of *Trachelomonas* and *Strombomonas* became very common or abundant in a number of ponds of north-east and south India, the meteorological and physico-chemical conditions under which they became dominant are available only for a few ponds. Nor were these studied in detail since the present account is more of a taxonomic nature.

Table 1 shows the conditions under which a number of *Trachelomonas* and *Strombomonas* species became dominant in 6 ponds. *Trachelomonas volvocina* which became very abundant in the Museum pond, Madras, and Chaudwar pond 6, Orissa, appeared to be favoured by moderate temperatures, high sunshine values and absence of rains, and higher nitrates than phosphates. High temperatures in summer, especially when the water level was low, was found to have an adverse effect on this organism since it migrated to the bottom layers during the bright hot hours of the day and back to the surface at night. With continuous rains obtaining in the winter monsoon months it became much less or disappeared altogether (also see Philipose 1940). *Strombomonas verrucosa* var. *zmiewika* and *S. verrucosa* var. *borystheniensis* on the other hand, seemed to be favoured by a certain amount of rainfall accompanied by lowering of temperature and slight increase in nitrates.

Moat extension at Cuttack and the swamp at Kausalyagang where organic matter was high due to the decomposition of weeds, nitrates and phosphates were high, with the latter higher, and the water level was low, several species of *Trachelomonas* and a few of *Strombomonas* occurred. Dhar's pond, Barrackpore, and Dwarapudi pond, Srikakulam, also showed a wide variety of *Trachelomonas* and *Strombomonas*. These 4 water areas had bright hours of sunshine, moderately high temperatures, no rainfall and phosphates higher than nitrates.

Six other ponds (not included in the table) worth mentioning are Stocking tank-4, Joyasagar, in which 8 species of *Trachelomonas* (including *T. volvocina* which was abundant) and *Strombomonas verrucosa* var. *conspersa* occurred on 4–6–1955; Gaurisagar tank, Joyasagar, with 14 species of *Trachelomonas* on 10–12–1965; Sagore Dutt pond-2, Kamarhati, in which 6 species of *Strombomonas* and

Table 1. Showing the meteorological and water conditions in 6 ponds in which *Trachelomonas* and *Strombomonas* dominated and the species concerned.

Water bodies and dates of collection	Dhar's pond Barrackpore 5–4–1949	Moat Extension Cuttack 11–4–1956	Tank 6 Chaudwar Farm 2–1–1953	Swamp Kausalyagang 10–4–1951	Dwarapudi pond Srikakulam 3–12–1954	Museum Pond Madras 28–2–1938	Museum Pond Madras 17–11–1938	Museum Pond Madras 15–4–1939	Museum Pond Madras 19–7–1939
Average sunshine hours (previous fortnight)	[Bright Sunshine]					10·5	9·7	7·9	4·4
Rainfall (cm) (previous fortnight)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	9·1	2·74
Water level (meters)	1·65	0·92	2·52	0·62	1·07	2·74	2·52	1·52	0·76
Turbidity (ppm)	50	80	30	60	72	20	28	40	45
Water temp. (°C)	29·4	32·5	28·1	37·4	31·2	29·0	26·4	27·5	29·0
pH value	8·2	7·1	7·9	7·0	8·4	7·4	7·2	7·0	7·2
Dissolved oxygen (ppm)	3·0	1·2	—	—	8·1	20·0	20·0	4·0	20·0
Oxidisable organic matter (ppm)	—	[high]	—	[high]	—	3·6	2·7	2·9	5·0
Total alkalinity (ppm)	282	128	92	32	132	183	138	162	192
Nitrates (ppm)	0·06	0·12	0·26	0·09	0·04	1·20	0·70	1·10	0·80
Phosphates-P04 (ppm)	0·36	0·24	0·11	0·12	0·74	Trace	0·01	0·015	0·02
Total iron (ppm)	—	—	—	—	—	0·5	0·2	0·3	0·5
Algal species									
Trachelomonas volvocina	c	vc	va	—	c	va	va	a	vr

T. varians	—	a	—	—	—	—	—	—	—
T. hispida var. *crenulatocollis*	—	—	rc	r	—	—	i	—	—
T. oblonga	—	—	—	—	rc	—	—	—	—
T. armata	—	vr	—	rc	—	—	—	—	—
T. scabra	r	—	—	—	rc	—	i	—	—
T. hexangulata	r	—	—	—	rc	—	—	—	—
T. similis	r	—	i	—	—	i	vr	—	—
Trachelomonas spp. (6 taxa)	—	—	—	vc	—	—	—	—	—
Strombomonas verrucosa var. *conspersa*	c	r	—	—	—	i	i	vr	vr
S. verrucosa var. *borystheniensis*	—	—	—	—	—	—	i	vc	r
S. verrucosa var. *zmiewika*	—	—	—	—	—	—	i	c	rc
S. napiformis var. *brevicollis*	—	—	—	—	—	—	—	vr	r
Strombomonas spp. (3 taxa)	c	—	—	r	rc	—	—	—	—

a, Abundant; c, common; r, rare; i, isolated; va, very abundant; vc, very common; rc, rather common; vr, very rare.

Trachelomonas scabra became dominant on 30–5–1950; pond No. 17, Jobra, Cuttack, in which *Strombomonas verrucosa* var. *conspersa* became abundant on 19–5–1955; pond 2, Birbati, Cuttack, with 9 species of *Trachelomonas* on 25–2–1957; and Thandankulam, Azhicode, which showed 7 species of *Trachelomonas* on 26–2–1949. Of these, except Sagore Dutt pond-2, all had total low alkalinity (50–70 ppm). Also, in cultures of silt from pond 16, Jobra, Cuttack, collected on 23–9–1951 and kept in the laboratory, 11 taxa of *Trachelomonas* appeared on 26–9–1951.

Thus, *Trachelomonas* and *Strombomonas* dominated in most ponds usually during December–May or early June when there was bright sunshine and no rainfall. Though total alkalinity seemed to be on the higher side (80–200 ppm or more) for *T. scabra* and most species of *Strombomonas* (e.g. *Strombomonas verrucosa* var. *zmiewika*, *S. gibberosa*, *S. napiformis* var. *brevicollis*, *S. praeliaris*, *S. tambowika*, *S. triquetra* var. *torta* and *S. reisii*), it was on the lower side (30–70 ppm) for most species of *Trachelomonas* (e.g. *T. lismorensis*, *T. costatus*, *T. mucosa*, *T. hispida* var. *crenulatocollis*, *T. armata*, *T. volzii* var. *cylindracea*, *T. pseudocaudata* var. *elongata*, *T. helvetica* and *T. nadsonii* var. *indica*). *T. volvocina* and *Strombomonas* var. *conspersa* seemed to tolerate a wider range of total alkalinity. Some ponds like Moat Extension, Cuttack, Birbati pond, Swamp, Kausalyagang and Thandankulam had also high organic matter due to the decomposition of weeds.

Incidentally, it may be mentioned that in British ponds, both Lund (1942) and Philipose (1948) observed the predominance of *Trachelomonas* spp. after the autumn leaf fall (usually October–November) when organic matter was maximum. Philipose also observed that high nitrates and phosphates coincided with the maximum of *Trachelomonas* in November.

4. Systematic account

Genus *Trachelomonas* Ehrenberg 1833

Single-celled and free swimming with delicate membrane, highly metabolic, and enclosed within a stiff and brittle envelope (lorica) of different shapes, the protoplast partially or completely filling the lorica; lorica spherical, subspherical, ovoid, cylindrical or spindle-shaped, with or without a collar at its opening (pore) and with or without a tail; pore with or without an annular thickening; lorica smooth or rugged, dentate or with spines; colourless (mostly when juvenile) or coloured yellow, brown or sometimes red; lorica rarely enclosed within a mucilaginous envelope; with a flagellum which is usually more than body length; vacuolar system as in *Euglena*; Chromatophores two to numerous and discoid, trough-shaped or parietal, rarely absent, and with or without pyrenoids which are often double-sheathed (diplopyrenoids) and sometimes projecting and button-like; paramylum bodies roundish or elongated, rarely ring-shaped or sometimes absent; eye-spot usually present, semi-spherical or streak-like and lying by the side of the reservoir. Mostly in standing freshwater, rarely in brackish water.

Key to the Indian species described

I. Lorica generally without a tail . . . Section I. *Rotundatae*
  (A) Lorica spherical to subspherical or compressed . . . . . . Subsection 1. *Sphaericae*

(1) Lorica spherical to subspherical and smooth . . . . . . Group Volvocinae
  (a) Lorica spherical, collar usually absent, very rarely present
    (i) Chromatophores 2 and with pyrenoids; 5–32 μm in diameter . . . 1. *T. volvocina*
    With a cylindrical partially depressed collar; 14–15 μm in diameter . . . . . . var. *planctonica*
    (ii) Chromatophores usually up to 10, rarely up to about 30, and without pyrenoids; 14–16 μm in diameter . . . . . . 2. *T. volvocinopsis*
  (b) Lorica spherical to broadly ellipsoid with depressed collar; chromatophores numerous and without pyrenoids; 22–27 μm in diameter . . .3. *T. varians*

(2) Lorica with small spines all over the surface or at one or both poles only . . . Group Spiniferae
  Lorica spherical and with small spines scattered at fair distances; 17–18 μm in diameter . . . . . . 4. *T. sparsesetulosa*

(3) Lorica spherical to subspherical and with diverse ornamentation . . . . . . Group Diversisculptae
  (a) Surface covered with close granulations or verrucae or granular pustules; lorica 18–34 μm . . . . . . 5. *T. verrucosa*
  Ornamentation in the form of circular to semi-circular tubercles; lorica 14–15·8 μm . . . . . . var. *macrotuberculata*
  (b) With prominent longitudinal ribs; 13–27 μm in diameter . . . . . . 6. *T. costatus*
  (c) Lorica with reticulate sculpturing; 14–15 μm in diameter . . . . . . 7. *T. assamensis*
  (d) Lorica spherical to slightly rectangular with sharp spiral striae to the left; 22–27 × 19–23 μm . . . . . . 8. *T. heduma*

(4) Lorica compressed along the longitudinal axis with one or both poles flattened . . . . . . Group Compressae
  Without any collar round the pore . . . Subgroup Mespiliformes
  (a) Both poles flattened
    (i) Lorica smooth, pore with annular thickening; 17–29 × 20–31 μm . . . . . . 9. *T. curta*
    (ii) Lorica with radially arranged spines; pore small and surrounded by a row of small spines; Lorica 20 μm; spines up to 4 μm . . . . . . 10. *T. lismorensis*
    One row of spines below the equator and above it longer, the rest shorter; spines up to 6 μm . . . . . . var. *biseriata*
  (b) Front end only flattened, membrane with prominent tubercles including 5 conical ones around the pore; lorica 19–24 × 20–22 μm . . . . . . 11. *T. tuberculata*
  Tubercles irregular in shape and arrangement; lorica 16·3 × 14·1 μm . . var. *irregularis* var. *nov.*

(B) Lorica elongate-ellipsoid to cylindrical or ovoid; with or without a short collar; diversely ornate, rarely smooth . . . . . . Subsection 2. *Ellipticae*
  (1) Lorica smooth or diversely ornate but not wrinkled or scrobiculate . . . . . Group Intermediae

(a) Lorica ellipsoid with front end sometimes slightly broadened . . . . . . Subgroup Oblongae

(i) Lorica smooth

($^+$) Lorica with or without a short collar and not enclosed within mucilage; 12–19 × 9–13 μm . . . . . 12. *T. oblonga*

Lorica subspherical to 4-cornered and with rounded edges; with wide depressed collar; 11–22 × 10–20 μm . . . . . . var. *australica*

($^{++}$) Lorica with short cylindrical collar and enclosed within mucilage; 44 × 33 μm . . . . . . 13. *T. mucosa*

Lorica spherical and smaller; 12 μm in diameter . . . . . . var. *sphaerica* var. *nov.*

Lorica subglobose and smaller; collar absent or reduced; 17–21·2 × 15·4–19·4 μm . . . . . . var. *subglobosa* var. *nov.*

(ii) Lorica ornamented

($^+$) Lorica subspherical to broadly ellipsoid and finely punctate or rarely with small tubercles; chromatophores 3–11 and with diplopyrenoids; 16–23 × 14–19 μm. 14. *T. intermedia*

Lorica larger (31·7 × 28·2 μm); chromatophores 10–11 . . . . . .var. *major* forma *nov.*

($^{++}$) Lorica small, broadly ellipsoid and with minute warts; pore without collar but with a crown of spines; 16·5–17 × 15–16 μm . . . . . . 15. *T. neotropica*

($^{+++}$) Lorica large and ellipsoid, densely covered with small warts; chromatophores many, without pyrenoids; 60–64 (–65) × 34–42 μm . . . . . . 16. *T. ovata*

(b) Lorica very much elongated and ellipsoid to cylindrical . . . . . . Subgroup Cylindricae

Lorica cylindrical with parallel sides, depressed collar and smooth; 16–20 × 8–10 μm . . . . . . 17. *T. cylindrica*

Without collar; 16–20 × 8–10 μm, var. *decollata*

(2) Lorica variable in shape and usually with spines all over, rarely at poles only . . . . . . Group Spiniferae

(a) Spines short, conical and numerous

(i) Lorica ellipsoid with spines all over, rarely at poles only or absent altogether; with or without collar; with 8–10 chromatophores having diplopyrenoids; 20–42 × 15–26 μm . . . . . 18. *T. hispida*

($^+$) Lorica with collar

(*) Lorica with spines

(×) Collar widening at pore; 10–13 discoid chromatophores without pyrenoids; 31·7 × 18·5 μm . . . . . . var. *crenulatocollis*

(× ×) Collar cylindrical; 24·6–26·4 × 17·6–19·4 μm . . . . . . fa *recta*

(**) Lorica smooth and narrower; chromatophores many and without pyrenoids; 37 × 14·5 μm. fa *glabra* fa *nov.*

($^{++}$) Lorica without collar

With uniform short spines but no punctae; pore with a crown of spines joined at the base or separate. (30 –) 37–40 × 19–21 μm . . . . . . var. *coronata*

(ii) Lorica more or less cylindrical with nearly parallel sides; chromatophores numerous and without pyrenoids; 30–35 × 19–22·5 μm . . . . . . 19. *T. allia*

(iii) Lorica ellipsoid with very fine spines uniformly distributed; without collar; pore with annular ring and very small teeth; 26·2 × 20·2 μm . . . . . . 20. *T. joysagarensis* sp. *nov.*

(iv) Lorica subspherical to ellipsoid with irregular rows of tubercles or short spines at both poles; pore without collar; 34–39 × 31–35 μm . . . . . . 21. *T. kelloggii*

(b) Spines short, obtuse and rod-like . . . . . . Subgroup Bacilliferae

Lorica elongate-ellipsoid or cylindrical; without collar; 35–40 × 33–38 μm . . . . . . 22. *T. bacillifera*

(i) Lorica smaller than in the typical species; 22–28 × 18–26 μm . . . . . var. *minima*

(ii) Spines smaller in number and irregularly disposed; (21 –) 27 × 22–30 μm . . . . . . var. *sparsispina*

(c) Spines conical, stout, scattered and of different lengths . . . . . Subgroup Crassispinae

(i) Lorica ellipsoid, spines short; pore without collar but often with several spines; 20–30 × 17–23 μm . . . . . . 23. *T. robusta*

Lorica obovoid, spines thicker with blunt ends, and closer; 45·4 × 24·2 μm . . . . . . var. *major* var. *nov.*

(ii) Lorica ellipsoid, with spines of varying lengths; pore with or without annular ring and collar; 38–55 × 30–39 μm . . . . . . 24. *T. superba*

With a crown of spines of equal length around pore and only a few spines in posterior half; 30·8 × 26·4 μm . . . . . . var. *coronata* var. *nov.*

(iii) Lorica ellipsoid, scrobiculate and densely covered with very stout prismatic spines; with or without collar; 40 μm . . . . . . 25. *T. horrida*

With a prominent cylindrical collar, stout membrane and reduced scattered spines; 37–39 (– 42) × 20–22 μm; collar 5·7–7·5 μm long . . . . . . var. *moenacanthum*

(d) Hind end of lorica with a varying number of stout spines . . . . . . Subgroup Armatae

(i) Lorica ellipsoid to ovoid; posterior spines usually recurved and convergent; pore with or without an annular ring and collar; 29–37 × 22–29 μm . . . . . . 26. *T. armata*

(+) Lorica larger (37–40–42 × 30–33 μm) and with one or more crowns of spines of variable number and size around the pore; hind end with long spines; 37–40 × 30–33 μm . . . . . . var. *steinii*

(++) Lorica ovoid with scattered short conical spines; anterior end with a number of medium-sized spines; hind end with stout spines of varying length; 37 × 33 μm . . . . . . var. *malabarica* var. *nov.*

(+++) Lorica ovoid with loose granulations and a crown of long spines at both poles; with very short collar with 4 dents;

46 × 35 μm . . . . . . var. *duplex*

Granulations closer; anterior and posterior spines longer and fewer, with the latter often lateral; 46–50 × 35·5–36·5 μm . . . . . f. *jorhatensis* forma *nov.*

(++++) Lorica often with flattened poles; anterior pole with short and posterior pole with long, stout spines; membrane thick and finely punctate; (39 –) 41–43 × 29–30·5 μm . . . . . var. *longa*

(+++++) Lorica more ovoid and with posterior end broader, and with long spines; entire surface also covered with widely placed short spines; 41–45 × 32–34 μm . . . . . . var. *longispina*

Entire surface with widely placed short spines and punctae; 42 × 31·7 μm . . . . . . f. *punctata* forma *nov.*

(ii) Lorica ellipsoid, rarely ovoid, with arched sides; posterior pole with a crown of thick straight or divergent long spines; entire surface with short rod-like obtuse spines or papillae; 36–40 × 27–28 μm . . . . . . 27. *T. dangeardiana*

(+) Hind end as in type; membrane otherwise smooth; 34–46 × 28–34 μm . . . . . . var. *glabra*

(++) Lorica more cylindrical to oblong with posterior spines slightly recurved; 43 × 28 μm . . . . . . var. *cylindrica* var. *nov.*

(3) Lorica ovoid, usually with spiral striae, sometimes with spines . . . . . . Group Helicoideae

Striae punctate; pore with depressed collar with free end wavy; 26 × 19 μm . . . . . . 28. *T. eurystoma*

Lorica cordate with broad pore; membrane smooth; 15–35 μm . . . . . . var. *nuda*

(4) Lorica elongate-ellipsoid with hind end sometimes conical; usually without collar; membrane with fine dents or scrobiculae . . . . . . Group *Scrobiculatae*

(a) Lorica ellipsoid with arched sides, finely and densely scrobiculate; two chromatophores with diplo-pyrenoids; (15·7 –) 19–22 × 14–16–19·8 μm . . . . . . 29. *T. zorensis*

(b) Lorica ellipsoid-cylindrical with arched or parallel sides; with fine scrobiculae; 10 chromatophores without pyrenoids; 22–30 × 12–19 μm . . . . . . 30. *T. abrupta*

(i) Lorica smaller (15·5–22 × 9–12 μm) and densely punctate . . . . . . var. *minor*

(ii) Lorica elongate-ellipsoid with arched sides; 20–30 × 14–18 (– 21) μm . . . . . . var. *arcuata*

(c) Lorica ellipsoid with curved sides; surface with small granules or fine pearls; 17–26 × 13–22 μm . . . . . . 31. *T. granulosa*

Lorica ellipsoid with coarse punctae or lens-like elevations; collar short and widened towards outside and tooth-like tubercles; 24·7 × 16–20 μm . . . . . . var. *crenulatocollis*

Lorica more or less obovate with scrobiculae less crowded; 28·2 × 17·6 μm . . . . . . f. *obovata* forma *nov.*

(5) Lorica variable in form with hind end sometimes pointed; membrane always rugged or wrinkled . . . . . . Group Scabrae

(a) Lorica ellipsoid with hind end sometimes pointed; with a wide depressed collar; membrane rugged but never with regular granulations; 20–33 × 15–20 μm . . . . . . 32. *T. scabra*

(i) With long, straight or bent collar; 22–33 × 16–19 μm; collar 6–7 × 3·5 μm . . . . . . var. *longicollis*

(ii) Lorica subspherical with front end sometimes narrower; collar cylindrical; 18–20 × 17–19 μm; collar 3–4 × 3–4 μm . . . . . . var. *coberensis*

(iii) Lorica ovoid or ellipsoid with posterior half often slightly twisted; pore with two lip-like ridges; 20–22 × 17–18 μm . . . . . . var. *labiata*

(b) Lorica ellipsoid with numerous broad-based lumps from its surface; 21 × 19·2 μm . . . . . . 33. *T. szabadosiana*

Smaller form with fewer lumps; 15·8–16·7 × 13·2 μm . . . . . . f. *minor* forma *nov.*

(C) Lorica very variable in form and always with a fairly high collar; surface smooth or sometimes punctate . . . . . . Subsection 3. *Ampulliformes*

(1) Collar straight . . . . . . Group Erectae

(a) Lorica spherical, subspherical, ovoid or ellipsoid . . . . . . Subgroup Subglobosae

Lorica subspherical and strongly punctate; pore with stumpy, conical, irregularly toothed collar, 19–30 × 17–20 μm . . . . . . 35. *T. planctonica*

Smaller form; 12·3 × 8·8 μm . . . . . . f. *minor* forma *nov.*

(b) Lorica pear-shaped . . . . . . Subgroup Pyriformes

With cylindrical collar surrounded by a characteristic membrane cone at base; 32 × 15 μm; collar 4 μm high . . . . . . 36. *T. volzii*

Collar stout and without basal membrane; 34–38 × 16 μm; collar 4–5 μm . . . . . . var. *cylindracea*

(c) Lorica elongate to cylindrical . . . . . . Subgroup Elongatae

(i) Lorica smooth

(+) Lorica ellipsoid-cylindrical with parallel sides and a cylindrical collar; 22–26 × 11–14 μm . . . . . . 37. *T. dubia*

Lorica broader; 24–27 × 16 μm . . . . . . var. *lata*

(++) Lorica nearly 6-sided in front view with median region parallel; collar cylindrical, sometimes with a membrane cone; 24–34 × 10–16 μm . . . . . . 38. *T. hexangulata*

(ii) Lorica ornamented

Lorica elongate-ellipsoid, hind end sometimes attenuated; irregularly ornamented; collar broad and conical; 30–39 (−42) × 20–24 μm; collar 7–11 μm at base and 4–6·5 μm at the opening . . . . . .39. *T. bulla*

(2) Collar bent or curved . . . . . . Group Incurvae

(a) Lorica ellipsoid to quadrangular; collar cut regularly; membrane smooth; 19–23 × 16–18·5 μm; collar 3–3·5 μm high . . . . . 40. *T. playfairii*

Membrane hyaline and smooth; lorica 18·7–33 × 15–15·5 μm . . . . . . var. *hyalina*

(b) Lorica ellipsoid to ovoid; margin of collar irregularly dentate; membrane punctate; 22–40 × 14–23 μm; collar 3–3·5 × 4·5–5·5 μm ...... 41. *T. similis*

(c) Lorica ellipsoid with a few rows of hemispherical granulations in posterior half; 26–33 × 16–24 μm; collar 4–5·7 × 4–5·7 μm ...... 42. *T. atrata*
Front half of lorica often smooth or with scattered granules; granules in hind half grouped in 2–4; 13–16 × 10·5–13 μm; collar 2–4 × 2 μm ...... var. *pustulosa*

(d) Lorica ellipsoid with irregular ridgelike elevations of different shapes, size and thickness; 16·5–20 × 14–16·5 μm; collar 2·5–3 μm ...... 43. *T. gregussii*

II. Lorica always with a tail ...... Section II. *Caudatae*

(A) Lorica ellipsoid or ovoid to elongate with a collar and tail distinct from body; ornamented ...... Subsection 1. *Colliferae*

(a) Membrane with spines ...... Group Spiniferae
Lorica elongate-ellipsoid and covered with dense short obtuse spines; tail stout and bifurcate at tip; chromatophores 8–10 (−12) and without pyrenoids; 41–43·5 × 22–23 μm ...... 44. *T. pseudocaudata*
Lorica longer and narrower; 54–55·5 × 18–18·4 μm ...... var. *elongata* var. *nov.*

(b) Membrane without spines but diversely ornamented ...... Group Diversiornatae
Lorica elongate-ellipsoid, usually with curved sides; membrane densely scrobiculate; collar variable, rarely with annular ring; tail variable; 34–43 × 18–22 μm ...... 45. *T. bernardinensis*

(B) Lorica attenuated towards hind end, with tail not distinct from body ...... Subsection 2. *Obattenuatae*
Lorica usually ellipsoid to ovoid with conical tail; pore without collar; membrane covered with spines; 38–42 × 17·5–20 μm ...... 46. *T. helvetica*

(a) More elongated and narrower than type; 40–55 × 15–15·6 μm ...... var. *elongata*

(b) Smaller than type and with rounded hind end; 30–35 × 14·5–15·7 μm ...... var. *cucurbita*

(C) Lorica spindle-shaped, small to very broad, thick, coloured and with spines .... Subsection 3. *Speciosae*
Lorica with arched sides and with sparsely arranged spines; tail usually pointed at tip; chromatophores numerous and without pyrenoids; 66·5 × 22·5 μm .... 47. *T. nadsonii*
Lorica with irregular little broad spines or punctae; neck enlarged and serrated at pore; 50–55 × 17 μm, neck 8–8·5 × 5–5·2 μm ...... var. *indica*
Lorica punctate to granulate; tail stouter with bifurcate ends; 59–61·6 × 17·6 μm ...... f. *kausalyagangensis* forma *nov.*

1. ***Trachelomonas volvocina* Ehr. 1833 (Figures 1a, b)**

Deflandre 1926, p 55, figures 1–6; Huber-Pestalozzi 1955, pp 251–52, figure 349.

Lorica spherical; pore with or without an annular ring, sometimes with a very much depressed collar; membrane smooth, deep yellow, yellowish brown or deep reddish brown; with two chromatophores, each with a projecting double-sheathed pyrenoid; flagellum (1·5) – 2–3 times body length; lorica 10–15·8 μm in diameter; pore 2·1–2·6 μm; eye-spot usually disc-like (rarely triangular).

Habitat: One of the most common species occurring in fresh-water ponds, with maximum frequency usually during the cool dry months of the year; Observed in abundance in locations 5b, 5c, 6, 6a, 28 (April), 61 (NP 1 and 4, May), 63 (NP 24, January), 78a and 151 (very common to very abundant, January–December); Rather common to common in locations 4, 5d, 19, 22, 46, 46a, 51, 88b, 88c and 109; stray to rare in locations 1a, 1b, 4a, 18, 19a, 28 (April–June, August), 29 (January to December), 32, 33 (April–June, September–November), 35, 36a, 37, 38, 40a, 44, 44a, 51a, 63 (NP 25–28, January–March, July–November), 72, 74–76, 79, 85, 88, 88a, 88b, 88e, 89, 90, 90a, 93, 97a, 99, 102, 126, 130–132, 135, 135a, 136, 137, 137b, 138, 143, 154, 157 and 159.

The usual range of dimensions of the species is 5–32 μm (see Deflandre 1926; Huber-Pestalozzi 1955).

Distribution in the Indian region: Burma (Skvortzov 1937, 13·6–14·2 μm in diameter, flagellum 1·7 μm; Skuja 1949, 9–30 μm); Madras (Philipose 1940, 10–10·7 μm); Bombay (Gonzalves and Joshi 1946; Kamat 1964, 12–18 μm); Hyderabad (Suxena 1955, 17–17·5 μm; Zafar 1959); Gujarat (Kamat 1962, 8–28 μm); Kolhapur (Kamat 1963, 18–21 μm); Rajasthan (Kamat 1967); Alibag (Kamat 1968a); Simla (Kamat 1968b); Cranganore, Kerala (Suxena *et al* 1973); Vidarbh (Kamat 1975); Nagpur (Kamat and Freitas 1976); Karnataka (Bongale and Bharati 1980; Hosmani and Bharati 1983; Hegde and Bharati 1986); Aurangabad (Ashtekar 1982); Kandesh, Maharashtra (Barhate and Tarar 1985); Assam, West Bengal, Bihar, Madhya Pradesh, Karnataka, Kerala and Tamilnadu (!).

var. *planctonica* Playfair 1921 (figure 1c)

Playfair 1921, p 129, plate 6, figure 12; Huber-Pestalozzi 1955, p 252, figure 353.

Lorica spherical and smooth, with a straight cylindrical collar two-thirds of which projects outside the surface and one-third inside the lorica; collar thick-walled with both ends thinner; diameter of lorica 14–15 μm, collar 2·2 × 3·2 μm.

Habitat: Stray in locations 5c, 63 (NP 24, November), 76 and 159.

It agreed well with Playfair's species measuring 15 μm with collar 2 × 3 μm.

Distribution in Indian region: Assam, Orissa and Tamilnadu (!).

2. *Trachelomonas volvocinopsis* Swirenko (figures 2a, b)

Deflandre 1926, p 58, figure 20; Huber-Pestalozzi 1955, p 253, figure 358: = *T. indica* Skvortzov 1937, p 77, plate 10, figure 13.

Lorica spherical, smooth and brown, 15–16 μm in diameter; chromatophores usually about 10, rarely up to 30 or more, discoid and without pyrenoids; eye-spot (reported as red and elongated) and flagellum (reported as about 3 times body length and up to about 40 μm) not observed.

Habitat: Stray in locations 5c, 22 and 92.

Huber-Pestalozzi treats Skvortzov's (1937) *T. indica* (18·7–23 μm with pore 1·7–1·9 μm) as synonymous to *T. volvocinopsis* since it differs only in the larger number (38) of chromatophores. Further, he states that Bourrelly (1952) has expressed the same opinion. In the author's material from Dum Dum (location 22) there were about 30 chromatophores while in the Joysagar (location 5c) and Kausalyagang (location 92) materials there were only about 9–10. Diameter of the species as given by Swirenko is 14–16 μm, 26 μm as given by Deflandre and 15 μm as given by Prowse (1962).

Distribution in Indian region: Burma (Skvortzov 1937, as *T. indica*) Aurangabad (Ashtekar 1982); Kashmir Valley (Compère 1983); Assam, West Bengal and Orissa (!).

3. *Trachelomonas varians* Deflandre 1924 (figures 3a, b)

Deflandre 1924, pp 1124–25, plate 10, figures 6–7; Deflandre 1926, p 58, figures 37–40, 42–44 and 47–50; Huber-Pestalozzi 1955, p 256, figure 366; = *T. volvocina* var. *subglobosa* Lemmermann 1913, p 146.

Lorica subspherical to broadly ellipsoid (slightly longer than broad) but never perfectly spherical, smooth and dark reddish brown; pore with an interior cylindrical tube 4·4–5·3 μm long; chromatophores numerous, discoid or polyhedral and without pyrenoids; eye-spot rod-like or discoid; flagellum about 1½ times body length; lorica 23–24 × 20·3–22 μm with pore 3·5–5·3 μm in diameter.

Habitat: Stray to very rare in locations 14, 29 (April–May), 33 (April–May, October), 44a, 63 (January, March, August and November), 76, 88d, 100a and 135a; rare in cultures of silt from location 61 (NP 16, September) and abundant in location 65 (April).

Usual dimensions as given by Deflandre (1926) are 22–27 × 19–23 μm, with cylindrical tube 4·8–9·5 μm.

Distribution in Indian region: Vidarbh, Maharashtra (Kamat 1975); Aurangabad (Ashtekar 1982); Karnataka (Hosmani and Bharati 1983); Kandesh, Maharashtra (Barhate and Tarar 1985); Assam, West Bengal, Madhya Pradesh, Orissa and Karnataka (!).

4. *Trachelomonas sparsesetulosa* Huber-Pest. 1955 (figure 4)

Huber-Pestalozzi 1955, p 257, figure 370

Lorica regularly spherical with a narrow pore having an annular thickening but without a collar; surface of lorica with fine spines (about 1·5–2 μm long) scattered at fair distances from each other; lorica light brown in colour and 16·7–17·6 μm in diameter. Internal contents not observed.

Habitat: Stray in location 76 (February).

The organism agreed well with Huber-Pestalozzi's species measuring 17–18 μm in diameter. The flagellum is described by Huber-Pestalozzi as 2½ times body length.

Distribution in Indian region: Orissa (!).

5. *Trachelomonas verrucosa* Stokes 1887

Deflandre 1926, p 61, figures 78–79; Huber-Pestalozzi 1955, p 261, figure 392.

Lorica spherical and thick, and ornamented with small semicircular granulations or warts; pore without annular thickening; dimensions 18–24 × 20–34 μm.

The typical species not observed by the author but is known from Hyderabad (Suxena 1955, 23–24 μm, pore 1·7–2·3 μm).

var. *macrotuberculata* Grandori (figure 5)

Huber-Pestalozzi 1955, p 261, figure 396.

Lorica spherical or nearly spherical, deep reddish brown with fairly large and conspicuous roundish to papilla-like tubercles which are dark brown in colour; pore with a slightly depressed collar; lorica 14–16·7 μm in diameter.

Habitat: Rather common in location 125 (February).

The organism agreed fairly well with Grandori's variety. However, Grandori has not given the dimensions.

Distribution in Indian region: Andhra Pradesh (!).

6. *Trachelomonas costatus* Philipose sp. *nov.* (figures 6a, b)

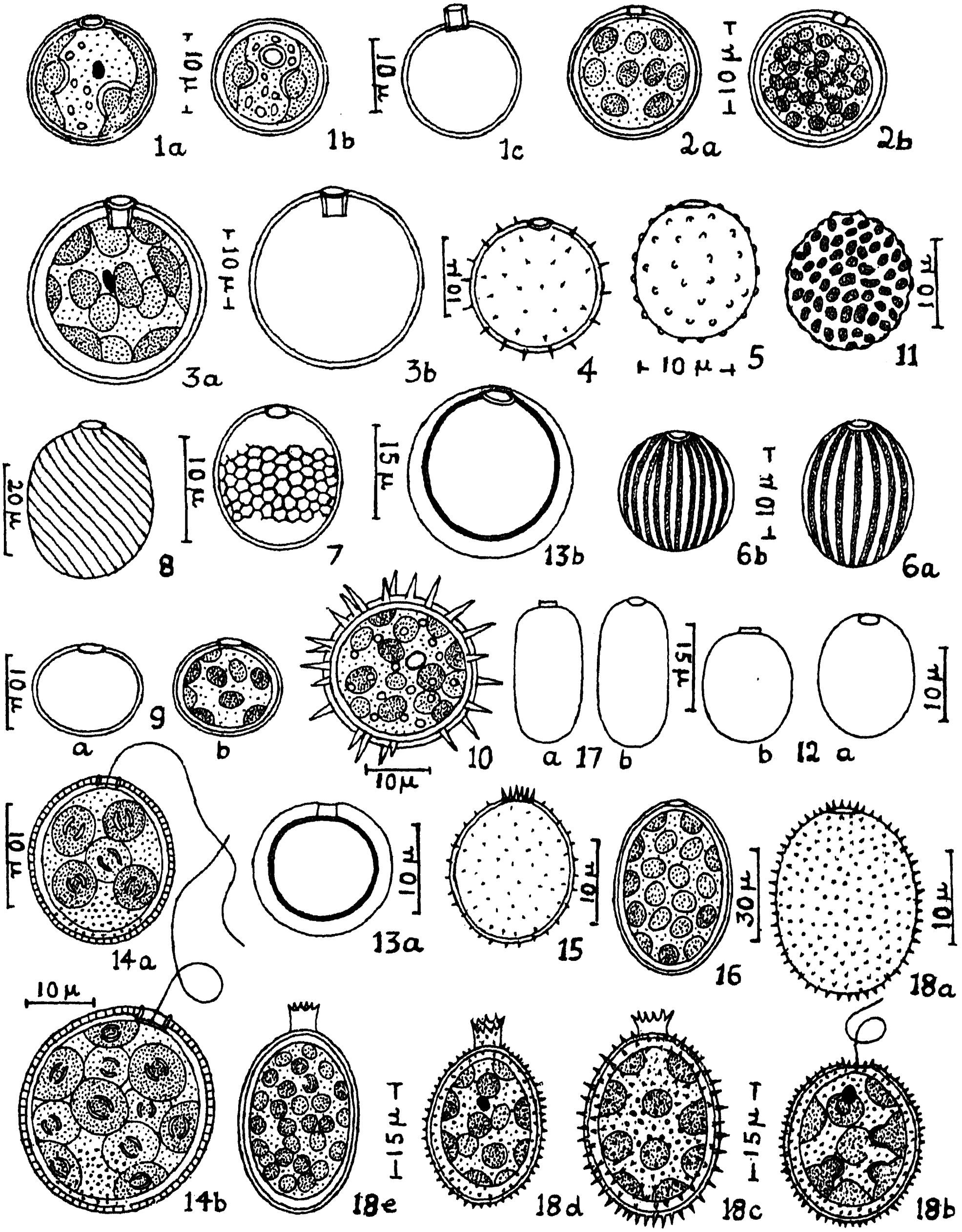
Lorica de more est sphaericalis vel aliquando ellipsoidea, plene fulva necnon aliquot plus minusve regulares, rara paululum internexas, longitudinales costas habens a vertice superiore ad verticem inferiorem procedentes, et in casibus individuis ab una vertice ad alteram radiare videntes; fistula circulo annulari; lorica 14·1–17·6 × 12·8–15·8 μm.

Habitatio: In planktone locationis 14 (mensibus Februario et Decembri) et in culturibus limi ex locatione 61 (NP 16, mense Septembri).

Typus: Figurae 6a, b.

Lorica usually spherical or sometimes ellipsoid, deep brown, and with a number of more or less regular, rarely slightly anastomosing, longitudinal ribs running from pole to pole, the ribs appearing to radiate from pole to pole in spherical individuals; pore with an annular ring; lorica 14·1–17·6 × 12·8–15·8 μm.

Habitat: In plankton from location 14 (February and December) and in cultures of silt from location 61 (NP 16, September).



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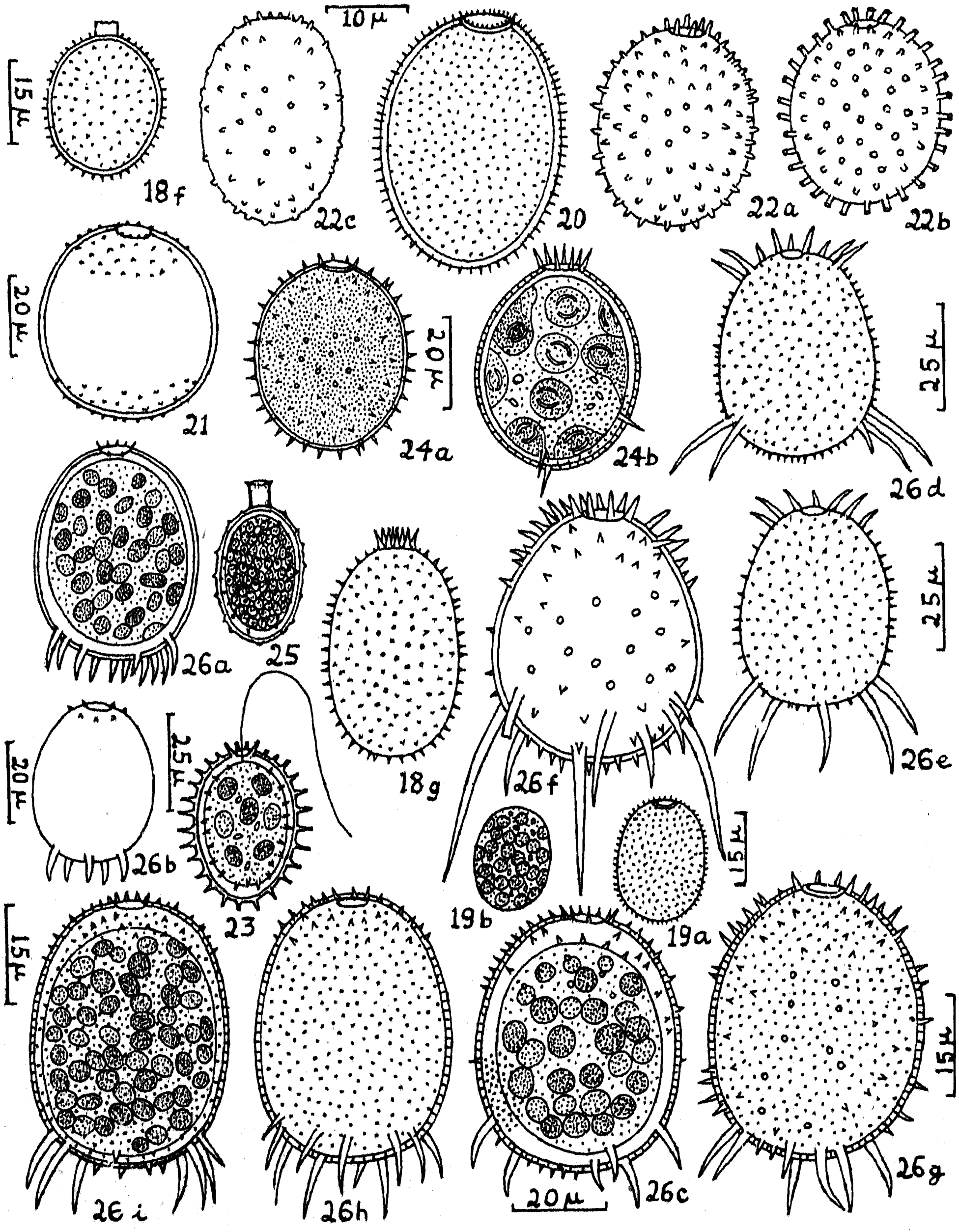

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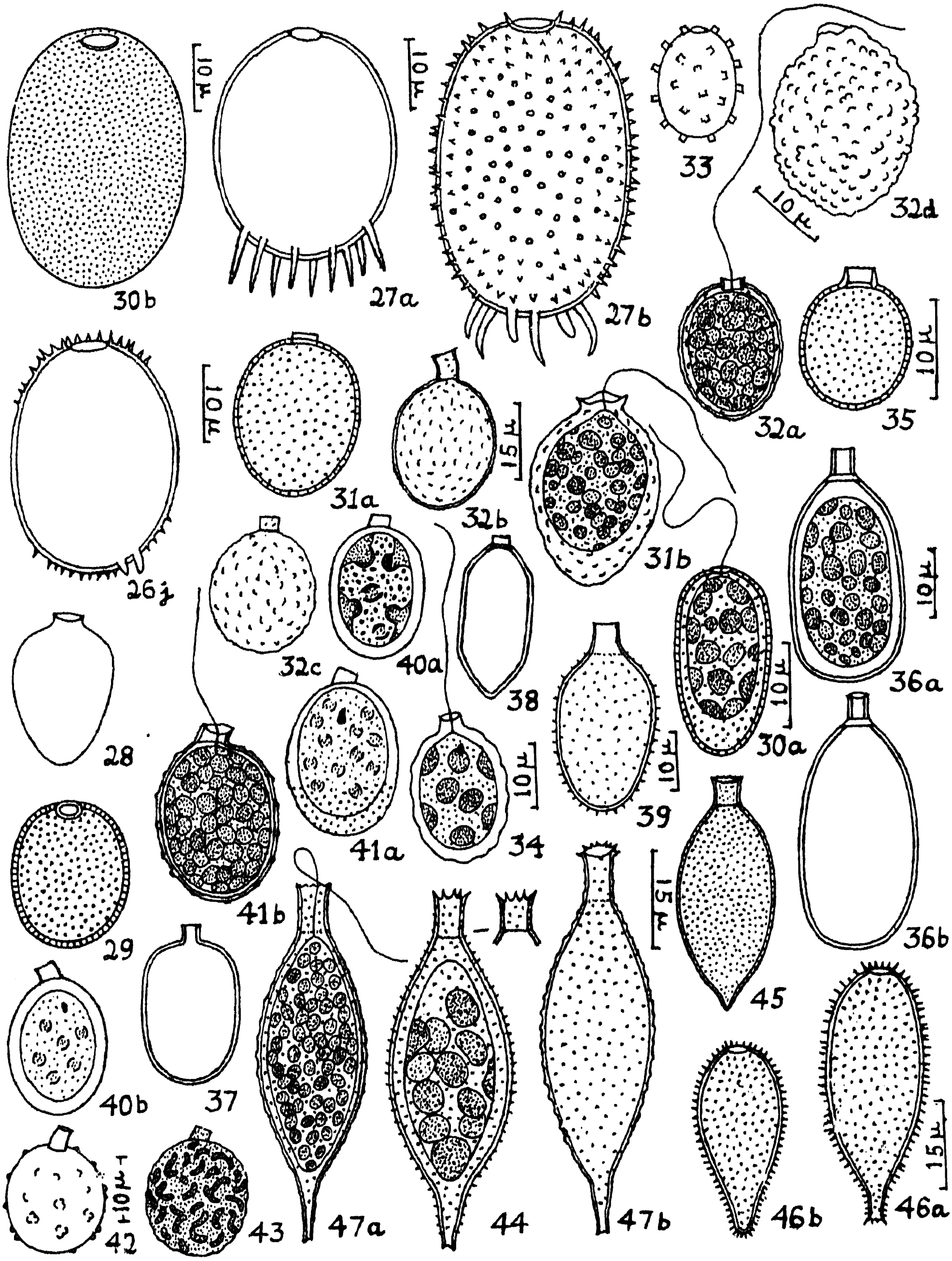

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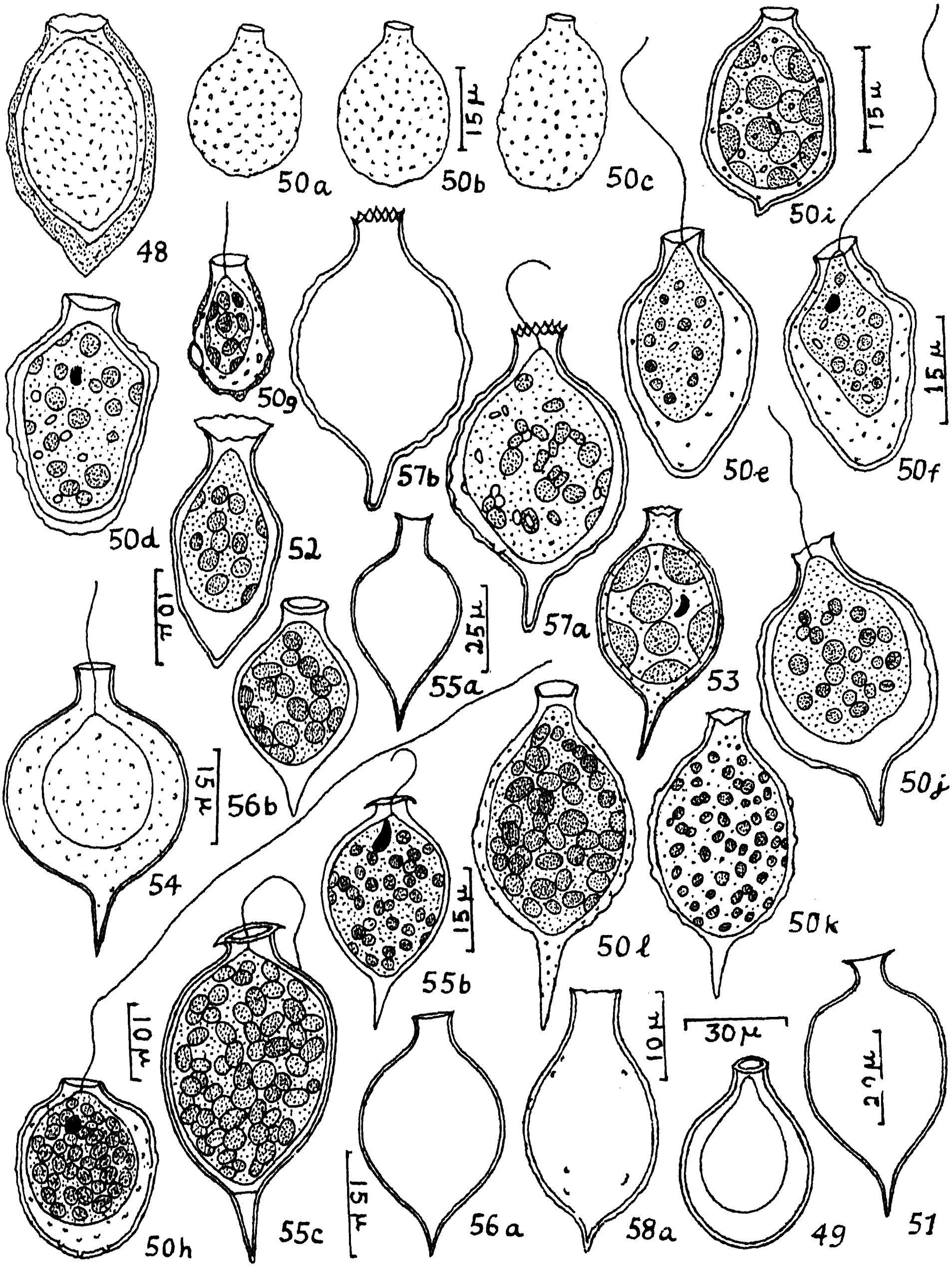

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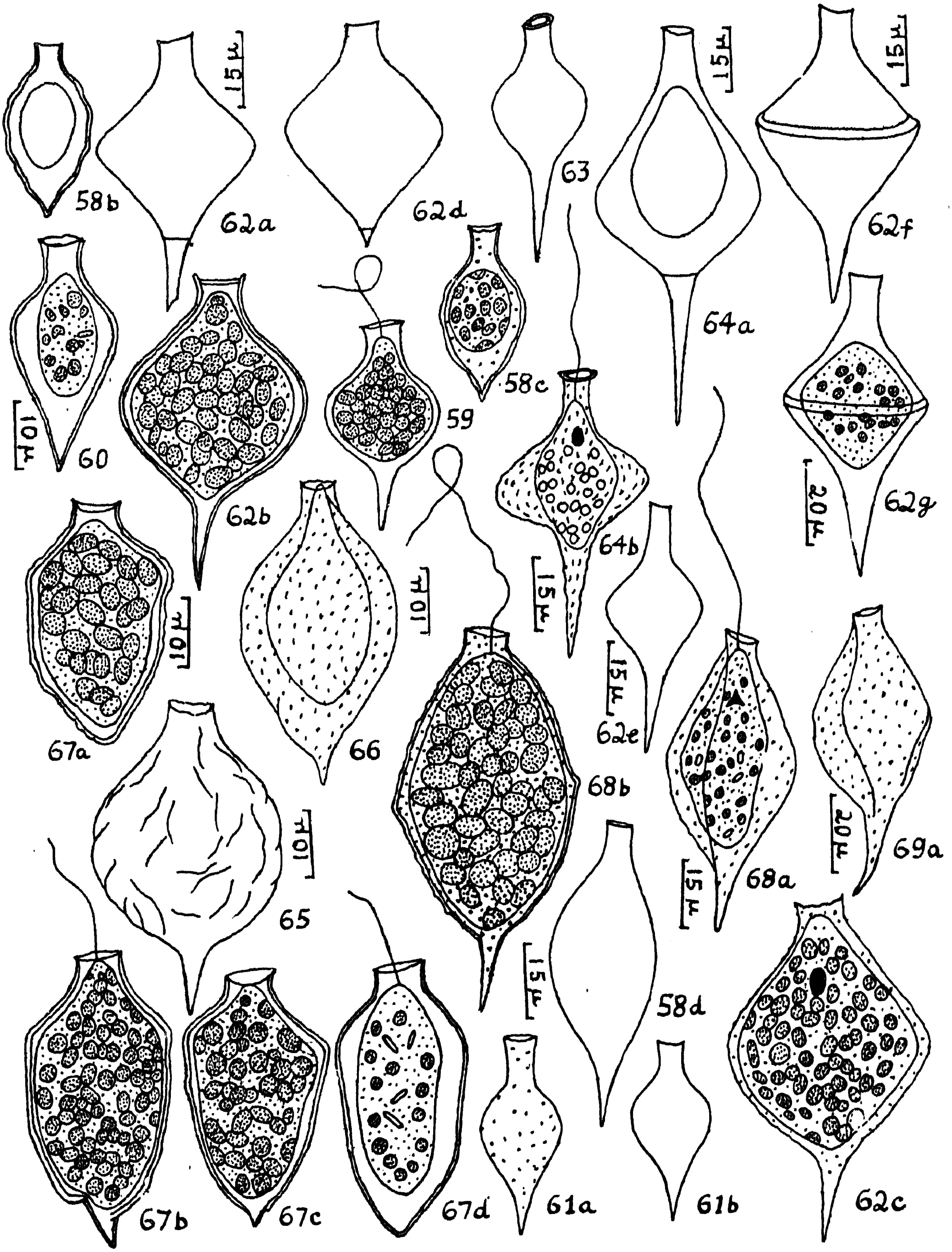

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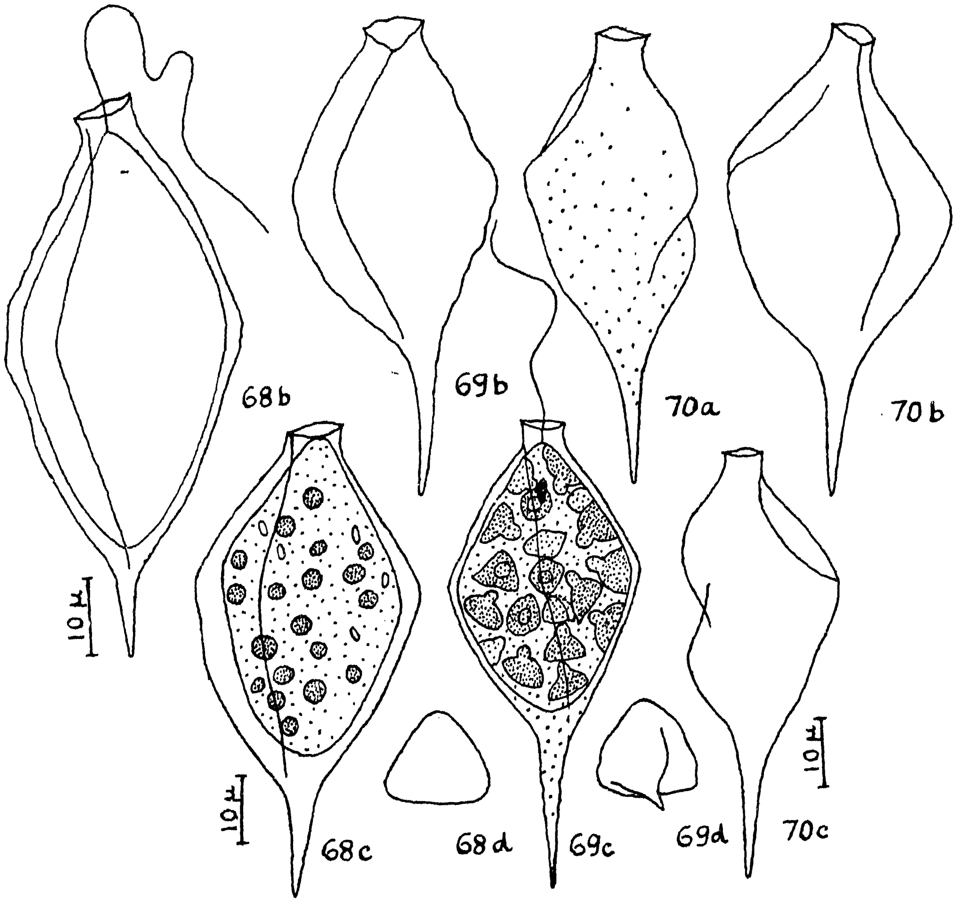

For caption, see p. 393.

Type: Figures 6a, b.

Distribution in Indian region: Assam and Orissa (!).

7. ***Trachelomonas assamensis*** **Philipose et Radhakrishnan sp.** ***nov.*** **(figure 7)**

Lorica est a forma subglobosa ad late ellipsoideam coloreque flavido-fulvo; superficies membrani ornamenta dense reticulateque; fistula parva sine annulari condensatione; lorica 14 × 12·2 μm; fistula 2·5 μm diametri.

Typus: Figura 7.

Habitatio: Abberrans in locatione 14 (Mense Februario).

Lorica subglobose to broadly ellipsoid and yellowish brown; surface of membrane with close reticulate ornamentation; pore small and without annular thickening; lorica 14 × 12·2 μm, pore 2·5 μm in diameter.

Type: Figure 7.

Habitat: Stray in location 14 (February).

The organism resembles *T. radiosa* var. *reticulata* Bourrelly (see Huber-Pestalozzi 1955, p 272, figure 439) in the reticulate nature of the wall but differs in the shape of the cell, the nature of the pore and the absence of longitudinal ribs. The name *T. reticulatus* would have been appropriate for this species but Klebs had used it for another species which Deflandre (1926) had reduced to a variety of *T. obovata*.

8. *Trachelomonas heduma* Conrad (figure 8)

Huber-Pestalozzi 1955, p 216, figure 415; *T. varians* Defl. f. *spiralis* Defl. 1926, p 58, figure 41.

Lorica usually spherical with diagonal unbranched spiral striae running to the left; pore with a circular thickening; cell contents not observed; lorica 23·6–14·5 × 20·3 μm.

Habitat: Stray in location 97a.

The organism agreed well with Conrad's species measuring 22–27 × 19–23 μm; Conrad has reported 6–10 discoid chromatophores without pyrenoids and an eye-spot in this species.

Distribution in Indian region: Orissa (!).

9. *Trachelomonas curta* Da Cunha emend. Defl. 1926 (figure 9a, b)

Huber-Pestalozzi 1955, p 269, figure 425; = *T. volvocina* var. *compressa* Drez. 1925, p 265, figure 2; = *T. volvocina* var. *compressa* emend. Defl. 1926, p 56, figures 17–19, 21, 26–30; Skvortzov 1937, p 76, plate 10, figure 12.

Lorica a flattened smooth deep brown sphere; both poles uniformly arched; pore with a light ring-like thickening; chromatophores about 8–9, discoid and without pyrenoids; flagellum and eye-spot not observed; lorica 11·4–14 × 13·4–15·8 μm with pore 1·5–2·2 μm.

Habitat: Stray in locations 22 (September) and 135a (October).

Deflandre (1926) and Huber-Pestalozzi (1955) give two size groups, viz. 20–29 × 22·5–31 μm and 17–18 × 20–21 μm. The author's specimens from West Bengal and Karnataka were nearer Skvortzov's (1937) Burmese organism which measured 13·6 × 15·3 μm with pore 2 μm in diameter. Huber-Pestalozzi gives the number of chromatophores as 10–12, flagellum 2 times body length and eye-spot rodlike.

Distribution in Indian region: Burma (Skvortzov 1937) and Aurangabad (Ashtekar 1982), both as *T. volvocina* var. *compressa* Drez. emend. Defl.; West Bengal and Karnataka (!).

10. ***Trachelomonas lismorensis*** Playfair 1915

Deflandre 1926, p 66, figure 107; Huber-Pestalozzi 1955, p 272, figure 441.

Lorica spheroid and flattened, circular in apical view and with radially arranged spines; pore small and surrounded by a few small spines; in lateral view subglobose with the anterior pole flattened and the posterior pole broadly rounded, with radial spines of which the equatorial ones are longer; lorica dark yellowish brown, 20 μm in diameter with the spines up to 4 μm long; pore 2 μm.

The typical species known only from Australia.

var. ***biseriata*** Playfair 1915 (figure 10)

Deflandre 1926, p 66, figure 108; Huber-Pestalozzi 1955, pp 272–73, figure 443.

Lorica spheroid with the front and hind ends markedly compressed, with some spines of which one row above the equator and another below markedly longer; the spines at the anterior and posterior poles as well as the ones at the equator shorter; chromatophores numerous and without pyrenoids; lorica brown in colour, and excluding spines 17·6 μm in diameter; spines up to 6 μm long; pore 1·5–1·75 μm.

Habitat: Stray in location 136a.

Playfair gives the diameter of the organism as 20 μm. The Azhicode specimen was slightly smaller and the wall of the lorica was fairly thick.

Distribution in Indian region: Kerala (!).

11. ***Trachelomonas tuberculata*** Middelhoek

Huber-Pestalozzi 1955, p 274, figure 447A.

Lorica nearly spherical, broadly ellipsoid or ovoid with the front end slightly flattened; membrane very thick, yellowish or reddish brown, beset with prominent, loosely distributed, somewhat irregularly formed tubercles of which 5 surrounding the pore somewhat pointed and conical; with or without a well depressed collar; flagellum about 3 times the diameter of the lorica; chromatophores two; dimensions 19–24 × 20–22 μm.

The typical species not known from India.

var. ***irregularis*** Philipose var. ***nov.*** (figura 11)

Varietas a species typica differens eo quod tubercula sunt irregularia magnitudinis, formae distributionisque, necnon densius composita; anulom quinque tuberculorum circa fistulam quoque absente; sunt vero duo parva plus minusve cuneiformia tubercula ad utraque latera fistulae disposita; lorica plene fulva; lorica 16·3 × 14·1 μm; fistula 4 μm.

Habitatio: In culturibus limi ex locatione 61 (NP 16, mense Septembri).

Differs from the typical species in the tubercles being irregular in size, shape and distribution, and more closely arranged; the circlet of 5 tubercles around the pore also absent, only two small somewhat conical tubercles flanking the pore, one on each side; lorica deep brown and 16·3 × 14·1 μm; pore 4 μm.

Habitat: In cultures of silt from location 61 (NP 16, September).

Though the slight flattening of the lorica at the anterior end characteristic of the typical species was not observed, the organism obviously belong to *T. tuberculata* because of the nature of the tubercles and other features.

Distribution in Indian region: Orissa (!).

12. *Trachelomonas oblonga* Lemm. 1899 (figure 12a)

Lemmermann 1910, p 524; 1913, p 147, figure 278; Deflandre 1926, p 69, figures 117–118 and 120–124; Huber-Pestalozzi 1955, pp 278–79, figure 459.

Lorica elongate-ellipsoid, pore with or without an annular thickening; sometimes with a markedly depressed collar; membrane smooth, yellowish brown or dark reddish brown; chromatophores not observed; lorica 16–17·6 × 12·3–13·1 μm.

Habitat: Stray in locations 29 (March), 63 (NP 38, September, December), 72, 108 and 117; rather common in location 109 (December).

The organism agreed fairly well with the typical species measuring 12–19 × 10–13 μm. Chromatophores reported as two and projecting pyrenoids (see Fott 1959, figure 209c).

Distribution in Indian region: Burma (Skvortzov 1937; Skuja 1949, 9–15 × 7–12 μm); Gujarat (Kamat 1962, 15–17 × 12–13 μm); Kolhapur (Kamat 1963, 12–14 × 9–10 μm); Vidarbh (Kamat 1975); Nagpur (Kamat and Freitas 1976); Aurangabad (Ashtekar 1982); Kodaikanal (Suxena 1983, 13–15 × 11–12 μm); Kandesh, Maharashtra (Barhate and Tarar 1985); West Bengal, Orissa and Andhra Pradesh (!).

var. *australica* Playfair 1915 (figure 12b)

Deflandre 1926, p 70, figures 125–126; Huber-Pestalozzi 1955, p 280, figure 462.

Lorica subspherical or more or less quadrangular with rounded corners and always with a wide depressed collar; membrane smooth, yellowish brown; lorica 16·7 × 12·7 μm with collar 1 × 3 μm.

Habitat: Stray in location 80a.

The organism agreed well with Playfair's variety measuring 11–22 × 10–20 μm with collar 1 × 3–6 μm (see Deflandre 1926).

Distribution in Indian region: Kolhapur (Kamat 1963); Vidarbh (Kamat 1975); Orissa (!).

13. *Trachelomonas mucosa* Swirenko

Deflandre 1926, p 72, figure 154; Huber-Pestalozzi 1955, p 283, figure 481.

Lorica ellipsoid and with a small cylindrical collar 3–4·5 μm high; membrane smooth and maroon in colour; lorica with a mucilaginous envelope 5 μm in thickness; flagellum 2–3 times body length; lorica 44 × 33 μm.

The typical species (known only from Russia) not observed in the author's collections.

var. *sphaerica* Phil. *et* Radhakr. var. *nov.* (figura 13a)

Varietas a species typica differens eo quod lorica est plus minusve sphaericalis necnon multo minoris magnitudinis; lorica mucosa 15 μm, non mucosa 12 μm diametri; collare 1·6 μm altitudinis et 1·8 μm latitudinis; membranum subrufum vertens ad flavum.

Habitatio: Abberrans in locatione 14 (Mense Februario).

Differs from the typical species in the lorica being more or less spherical and much smaller in size; lorica with mucus 15 μm in diameter, without mucus 12 μm; collar 1·6 μm high and 1·8 μm broad; membrane reddish brown.

Habitat: Stray in location 14 (February).

Distribution in Indian region: Assam (!).

var. *subglobosa* Phil. *et* Radhakr. var. *nov.* (figura 13b)

Varietas a species typica differens eo quod lorica est minor et subglobosaque, collari vere absente; fistula habet formae anularis crassitudinem; membranum loricae subfufum vertens ad flavum coloris; lorica mucosa 19·4–24·7 × 18·8–21·2 μm; non mucosa 17·0–21·2 × 15·4–19·4 μm; fistula 3·5–5·3 μm diametri.

Habitatio: Rara in locatione 14 (mense Februario).

Differs from the typical species in the lorica being smaller and subglobose, and a collar is absent; pore with a rim; lorica membrane reddish brown; lorica with mucus 19·4–24·7 × 18·8–21·2 μm, without mucus 17–21·2 × 15·4–19·4 μm; pore 3·5–5·3 μm in diameter.

Habitat: Rare in location 14 (February).

Distribution in Indian region: Assam (!).

14. *Trachelomonas intermedia* Dangeard 1902 (figure 14a)

Deflandre 1926, p 71, figures 146–47, 157–58 and 160; Huber-Pestalozzi 1955, p 280, figure 467; Hortobágyi 1963, p 367, figures 872–880.

Lorica subspherical or broadly ellipsoid, yellowish to deep brown and finely punctate; pore with a ring-like thickening; chromatophores 4–5 and with double-sheathed pyrenoids; flagellum about 2-times body length; lorica 16·7–17·6 × 13–14 μm.

Habitat: Rare in location 151 (November) occurring as a scum along with other algae.

The usual dimensions given by a number of authors (see Deflandre 1926) are 16–23 × 14–19 μm and the number of chromatophores 4–5. Prowse (1959) gives the dimensions as 18–20 × 15–17 μm and the number of chromatophores as several and in his figure j of plate 1, 8 are indicated. Hortobágyi (1963) gives the number of chromatophores as 3–11. Further, according to him, the ornamentation of the lorica could either be in the form of punctae or larger elevations or tubercles.

Playfair's (1915) *Trachelomonas granulosa* var. *subglobosa* (see Deflandre 1926, p 95, figure 403; Huber-Pestalozzi 1955, p 322, figure 642) in part, with lower dimensions of 19 × 17 μm is in all probability only a *T. intermedia* (also see below).

Distribution in Indian region: Burma (Skvortzov 1937; Skuja 1949); Hyderabad (Suxena 1955, 20–21 × 17–17·5 μm); Vidarbh (Kamat 1975); Aurangabad (Ashtekar 1982); Kashmir Valley and Ladak (Compère 1983); Tamilnadu (!).

forma *major* Philipose f. *nov.* (figura 14b)

Forma a species typica differens ob magnitudinem majorem (31·7 × 28·2 μm); lorica sphaericalis necnon subrufa vertens ad flavum coloris; membranum habet punctas prominentes seu granulationes; fistula in formae anularis crassitudine; chromatophores 10–11 necnon cum pyrenoidis dupliciter vaginatis; flagellum circa unam et dimidiam partem longitudinis corporis.

Habitatio: Aberrans in locatione 33 (mense Maio).

Differs from the typical species in its larger size (31·7 × 28·2 μm); lorica subspherical and reddish brown; membrane with prominent punctae or granulations; pore with ring-like thickening; chromatophores 10–11 and with double-sheathed pyrenoids; flagellum about 1½ times body length.

Habitat: Stray in location 33 (May).

Playfair's *Trachelomonas granulosa* var. *subglobosa* in part, with dimensions of 28 × 26 μm, could probably be referred to this variety. In Playfair's variety the nature of the chromatophores is not known but in most other respects it agrees with the author's material.

Distribution in Indian region: West Bengal (!).

15. *Trachelomonas neotropica* Balech. 1944 (figure 15)

Huber-Pestalozzi 1955, p 286, figure 487A.

Lorica broadly ellipsoid with the hind end sometimes slightly narrowed, and covered with very small hyaline warts which are often visible only under high magnifications; pore without a collar but with a crown of 10–12 closely arranged sharply pointed spines (up to about 2 μm long) which are usually parallel or sometimes divergent; lorica 16·7–17·6 × 14·9–15·8 μm.

Habitat: Stray in locations 77 and 88.

The dimensions given by Balech are 16·5–17 × 15–16 μm with the pore 2–2·5 μm wide.

Distribution in Indian region: Orissa (!).

16. *Trachelomonas ovata* Roll (figure 16)

Huber-Pestalozzi 1955, p 286, figure 487B.

Lorica broadly ovoid or ellipsoid with the front and hind ends broadly rounded; without collar; membrane deep orange brown and slightly thickened at the opening of the pore; surface of lorica densely covered by very fine short rod-like spinules; lorica 59·8–60·7 × 40 μm with pore 6 μm in diameter; chromatophores numerous, discoid and without pyrenoids. Eye-spot, flagellum and paramylum bodies not observed.

Habitat: Stray in location 20.

The organism agreed well with Roll's species measuring 60–64 × 34–42 μm with pore 6–7·2 μm in diameter.

Distribution in Indian region: Assam (!).

17. *Trachelomonas cylindrica* Ehr. Sec. Playfair (figure 17a)

Deflandre 1926, p 75, figures 170–171; Huber-Pestalozzi 1955, p 290, figure 502.

Lorica cylindrical with the sides parallel, posterior end broadly rounded with anterior end somewhat flattened; pore with a depressed collar; lorica smooth and 18 (including collar) × 8·3 μm with collar 1 × 2·5–3 μm.

Habitat: Stray in location 5c.

The organism agreed with Playfair's species measuring 16–20 × 8–10 μm with collar 1 × 3 μm.

Distribution in Indian region: Burma (Skvortzov 1937); Himachal Pradesh (Kamat 1968a) and Assam (!).

var. *decollata* Playfair 1915 (figure 17b)

Deflandre 1926, p 75, figures 172, 182–184; Huber-Pestalozzi 1955, p 290, figure 503; = *T. euchlora* var. *parvula* Conrad.

Differs from the typical species in the absence of a collar; lorica smooth, deep yellowish brown and 19 × 8·8 μm.

Habitat: Stray in locations 5c, 72, 76, 99a, 137 and 160a.

Dimensions given by Playfair for this variety are more or less the same (16–20 × 8–10 μm).

Distribution in Indian region: Simla (Kamat 1968a); Vidarbh (Kamat 1975); Assam, Orissa, Kerala and Tamil Nadu (!).

18. ***Trachelomonas hispida*** **(Perty) Stein emend. Defl. 1926 (figures 18a–b)**

Deflandre 1926, pp 77–78, figures 202–203, 207–208 and 227; Huber-Pestalozzi 1955, p 295, figure 520; = *T. hispida* (Perty) Stein 1878; Lemmermann 1910, p 526, figures 14–15 (p 517).

Lorica ellipsoid and usually covered densely with short conical pointed spines but sometimes the spines are irregularly distributed or they may be absent here and there; pore with or without an annular thickening and sometimes with a few long spines around the pore; with or without a very much depressed collar; membrane finely punctate or sometimes apparently smooth and yellowish or reddish brown; chromatophores 8–10 and with double-sheathed pyrenoids; flagellum usually about 1½–2 times body length, rarely shorter; eye-spot disc-like; lorica 20–26·3 × 15·8–22 μm.

Habitat: A wide spread species observed in locations 1a, 5b–d (June), 6, 6b, 28 (rather common, April), 32, 33, 37a, 40a, 46, 46a, 48, 52a, 56a, 61 (NP 5, common-May), 63 (NP 24, 26, 29, 31, 32, 34, 36- January–March and July–December), 68, 74, 75, 80a, 87, 88d, 90, 93, 93a (NP 5- November), 97, 100a, 106, 121, 136a, 136c, 137a, 137b, 139, 147, 148, 151 (very common, November), rare to rather common (December).

Usual dimensions of this species is 20–42 × 15–26 μm. However, Bhatia (1930) gives them as 74 × 34 μm.

Distribution in Indian region: Kashmir (Bhatia 1930); Burma (Skvortzov 1937; Skuja 1949); Madras (Philipose 1940); Maharashtra (Kamat 1963, 18–20 × 12–16 μm; 1964, 21–24 × 19–21·5 μm; 1968; 1975); Himachal Pradesh (Kamat 1968a); Hyderabad (Venkateswarlu 1976); Karnataka (Bongale and Bharati 1980, in cultivated soils), Bijapur, Karnataka (Hegde and Bharati 1983); Kashmir and Ladak (Compère 1983); Assam, West Bengal, Bihar, Madhya Pradesh, Orissa, Andhra Pradesh, Kerala and Tamil Nadu (!).

var. ***crenulatocollis*** **(Muskell) Lemm. 1910 (figures 18c, d)**

Lemmermann 1910, p 526; 1913, p 150; Deflandre 1926, p 78, figure 228; Huber-Pestalozzi 1955, p 296, figure 526.

Lorica as in the typical species or as in variety *coronata* (see below) but the pore always provided with a cylindrical collar which is slightly widened towards the free end and is provided with teeth or spines; lorica (excluding collar) 26·4–31·7 × 19·4–20·3 μm, collar 3·5–5·3 × 3·5–5·3 μm; chromatophores discoid, numerous and without pyrenoids; eye-spot discoid.

Habitat: Rather common in location 78 (January); rare in location 92 (April); stray in location 64 (April); and rare in cultures of silt from location 61 (NP 16, September).

Distribution in Indian region: Orissa (!).

forma *glabra* Philipose f. nov. (figura 18e)

Forma a var. *crenulatocollis* dicta differens eo quodlorica est perfecte glabra pauloque angustior (31·7 × 14·4 μm, collare vero 5·3 × 5·3 μm); collare cylindricale in forma habens dentes tenues ad finem solutum; chromatophora sunt parvae, discoideae necnon numerosae sine pyrenoidis.

Habitatio: Aberrans in loco 92.

Differs from var. *crenulatocollis* in the lorica being perfectly smooth and slightly narrower (31·7 × 14·4 μm with collar 5·3 × 5·3 μm); collar cylindrical with fine teeth at free end; chromatophores small, discoid and numerous without pyrenoids.

Habitat: Stray in location 92.

Distribution in Indian region: Orissa (!).

forma *recta* Deflandre 1926 (figure 18f)

Deflandre 1926, p 78, figures 204–205 and 212; Huber-Pestalozzi 1955, p 296, figure 527.

Differs from var. *crenulatocollis* in the collar being perfectly cylindrical; lorica 24·6–26·4 × 17·6–19·4 μm, and with spines and punctae.

Habitat: Stray in locations 5d (April), 63 (November), 88, 102, 117 and 118.

Distribution in Indian region: Maharashtra (Kamat 1975); Karnataka (Bongale and Bharati 1980); Assam, Orissa and Andhra Pradesh (!).

var. *coronata* Lemmermann (figure 18g)

Lemmermann 1913, p 150; Deflandre 1926, p 78, figures 220–221; Huber-Pestalozzi 1955, p 296, figure 525.

Lorica usually slightly narrower than in the typical species and with dense short spines of uniform length; pore with a crown of slightly more robust spines which are distinct or joined at the base; lorica deep brown and 30·8 × 17·6 μm without spines, and with spines 36·8 × 20·3 μm.

Habitat: Rather common in location 78a; stray to rare in locations 5c, 22, 23, 76, 100a, 102, and in cultures of silt from location 61 (NP-16, September).

Distribution in Indian region: Maharashtra (Kamat 1975); Karnataka (Hegde and Bharati 1986); Assam, West Bengal, Orissa and Andhra Pradesh (!).

19. *Trachelomonas allia* Drezepolski 1925 emend. Defl. 1926 (figure 19a, b)

Deflandre 1926, p 79, figures 233 and 238; Huber-Pestalozzi 1955, p 297, figure 532; = *T. allia* Drezepolski 1925, p 259, p 71; Prowse 1958, p 180, figure 6y.

Lorica cylindrical-ellipsoid with broad and uniformly rounded poles and parallel sides, and about 1/3 times longer; densely covered with short, conical and pointed spines; pore without collar; membrane deep reddish to yellowish brown; lorica 26·3 × 19·8 μm.

Habitat: Stray in location 19 (April).

The alga agreed in all respects with Drezepolski's species except in size. Drezepolski gives the dimensions as 30–35 × 19–22·5 μm, whereas Prowse gives them as 45–48 × 30 μm.

Distribution in Indian region: Gujarat (Kamat 1962, 28–30 × 13–16 μm); Vidarbh (Kamat 1975); Aurangabad (Ashtekar 1982); Assam (!).

20. *Trachelomonas joysagarensis* Philipose *et* Radhakrishnan sp. *nov.* (figure 20)

Lorica est ellipsoidea habetque latera paululum arcuata necnon membranum crassum fulvi coloris denseque cooperto parvis, uniformibus acutisque spinis; collari absente; fistula satis magna habens anulum crassum refertum brevibus, tenuibusque dentibus regulariter dispositis; lorica 26·5 × 20·2 μm; fistula 4·7 μm diametri.

Typus: Figura 20.

Habitatio: Aberrans in locatione 14 (mense Februario).

Lorica ellipsoid with slightly arched sides and with thick brown membrane which is densely covered with uniform pointed spines; without a collar; pore fairly large and with a thickened ring provided with short fine regularly arranged teeth; lorica 26·5 × 20·2 μm; pore 4·7 μm in diameter.

Type: Figure 20.

Habitat: Stray in location 14.

Distribution in Indian region: Assam (!).

21. *Trachelomonas kelloggii* Skv. emend. Defl. 1926 (figure 21)

Deflandre 1926, p 87, figures 290–294 and 296; Huber-Pestalozzi 1955, p 300, figure 543.

Lorica subspherical or broadly ellipsoid with both poles provided with somewhat irregular rows of tubercles which are sometimes in the form of papillae or short spines particularly at the hind end where they are often pointed. The ornamentation sometimes reduced to simple granules or absent altogether; pore always without a collar and with or without an annular ring which is smooth or sometimes with conical tubercles which are low and well or ill developed; membrane fairly thick with fine dense punctae, and deep yellowish brown; lorica 36·7–37·5 × 32·9–33·8 μm with pore 7·5 μm in diameter.

Habitat: Very rare in location 14.

The usual dimensions of this species are 34–39 × 31–35 μm without spines (see Deflandre 1926; Huber-Pestalozzi 1955), but Hortobágyi gives them as 22·5 × 20 μm with pore 3 μm.

Distribution in Indian region: Assam (!).

22. *Trachelomonas bacillifera* Playfair 1915

Deflandre 1926, p 82, figure 255; Huber-Pestalozzi 1955, p 302, figure 553.

Lorica subspherical and densely covered with rod-like obtuse spines; membrane always deeply coloured reddish brown or yellowish-reddish brown; pore relatively narrow and without collar; lorica 35–40 × 33–38 μm (without spines).

The typical species not observed by the author, but reported by Kamat (1975) from Maharashtra.

var. *minima* Playfair 1915 (figures 22a, b)

Deflandre 1926, p 82, figures 244 and 254; Huber-Pestalozzi 1955, p 303, figure 556.

Lorica subspherical or elongate-ellipsoid and much smaller than the typical species; dimensions 23·8–25·2–27·5 × 17·6–19·4 μm; spines up to about 2 μm.

Habitat: Rare in location 14 and stray in location 48.

Dimensions given by Deflandre are 22–28 × 18–26 μm and 29 × 26 μm, the latter after Playfair.

Distribution in Indian region: Hyderabad (Suxena 1955, 23–24 × 18–19·5 μm); Maharashtra (Kamat 1975); Assam and Orissa (!).

forma *sparsispina* Deflandre 1926 (figure 22c)

Deflandre 1926, p 82, figure 253; Huber-Pestalozzi 1955, p 303, figure 557; = *T. hispida* var. *niezabitowskii* Drezepolski 1925, p 262, figure 53.

Differs from var. *minima* Playfair in the number of spines being much less and in their irregular disposition; lorica 21·1 × 16·7 μm.

Habitat: Very rare in location 14.

It is slightly smaller than Deflandre's form which measures 27 × 22 or 25 × 20 μm, the latter after Drezepolski. The breadth in Drezepolski's material given by Deflandre as 30 μm is probably a printing error which has been repeated by Huber-Pestalozzi 1955.

Distribution in Indian region: Assam (!).

23. *Trachelomonas robusta* Swirenko emend. Defl. 1926

Deflandre 1926, p 84, figures 257–260; Middelhoek 1951, pp 234–235, plate 5, figures 59–60; Huber-Pestalozzi 1955, p 305, figure 568.

Lorica ellipsoid with short robust conical distantly placed (2–4 in 10 μm) spines; pore without a collar but often with some spines of even length or longer than the rest of the spines and divergent or not; membrane yellowish to deep brown and smooth or finely punctate; dimensions 20–30 × 17–22 μm; spines up to 3·3 μm long.

The typical species not observed by the author but reported from Vidarbh (Kamat 1975), Karnataka (Bongale and Bharati 1980; Hosmani and Bharati 1983) and Aurangabad (Ashtekar 1982).

var. *major* Philipose *et* Radhakrishnan var. *nov.* (figure 23)

Varietas a species typica differens ob magnitudinem majorem formam obovoideam loricae, robustiores obtusiasque spinas arctius (3–4 in 10 μm) magisque regulariter dispositas; spinis vero mediis circa fistulam longissimis; fistula sine collari, ad latera vero habens quattuor divergentes spinas; lorica sine spinis 45·4 × 24·2 μm, cum vero spinis 52·8 × 33 μm; spinae 1·8–4·4 μm longitudinis.

Habitatio: Aberrans in locatione 14.

Differs from the typical species in its larger size, the obovoid shape of lorica, the more robust and obtuse spines which are arranged closer (3–4 in 10 μm) and more regularly, with the median spines and those around the pore longest; pore without a collar but flanked by about 4 divergent spines; lorica without spines 45·4 × 24·2 μm, and with spines 52·8 × 33 μm; spines 1·8–4·4 μm long.

Habitat: Stray in location 14.

In the typical species also the lorica is sometimes slightly obovoid (see figure 568b in Huber-Pestalozzi after Middelhoek 1948) but the nature and arrangement of the spines and the size of the lorica are different in the present organism. The chromatophores appeared to be 7, discoid and without pyrenoids and the flagellum more than body length as shown by Middelhoek. However, the discoid eye-spot shown by Middelhoek was not observed.

Distribution in Indian region: Assam (!).

24. *Trachelomonas superba* Swirenko emend. Defl. 1926 (figure 24a)

Deflandre 1926, p 81, figures 261–262, 265–269 and 273; Huber-Pestalozzi 1955, p 306, figure 569.

Lorica ellipsoid with robust conical spines of variable length at fair distances (about 2–4 in 10 μm); pore with or without an annular ring but sometimes with a depressed dentate collar; membrane fairly thick, finely punctate, reddish brown or deep yellowish brown; lorica (excluding spines) 40 × 32·5 μm; cell contents not observed.

Habitat: Stray in location 63 (NP 26 and 36, March and September).

The usual dimensions of the lorica (excluding spines) are 38–55 × 30–39 μm and including spines 51 × 37 μm (see Deflandre 1926). Cell with discoid chromatophores and large stigma according to Swirenko. Presence or absence of pyrenoids not known.

Distribution in Indian region: Burma (Skvortzov 1937, 34–56·1 × 28–32 μm; Skuja 1949, 38 × 33 μm with pore 8 μm wide); Maharashtra (Kamat 1963, 35–40 × 25–28 μm; 1975); Kodaikanal, Tamilnadu (Suxena 1983, 49–51 × 36–37·5 μm); Orissa (!).

var. *coronata* Philipose var. nov. (figure 24b)

Varietas a species typica differens eo quod spinae super loricam restricta sunt ad coronam circiter 7 paululum divergentium spinarum plus minusve uniformium longitudinis circa fistulam necnon ad fere paucas spinas longitudinis variabilis in dimidia parte posteriori, pauloque magnitudinis minoris; lorica plene flava membranumque valde punctatum; chromatophora circiter 8–10, discoideae dupliciterque pyrenoidis vaginatis; lorica 30·8 × 26·4 μm; spinae 7–9 μm longitudinis.

Habitatio: In limi culturibus ex locatione 61 (NP 16, mense Septembri).

Differs from the typical species in the spines on the lorica being restricted to a crown of about 7 slightly divergent long spines of more or less uniform length around the pore and to a few spines of variable length in the posterior half and the slightly smaller size; lorica dark brown, membrane strongly punctate; chromatophores about 8–10, discoid and with double-sheathed pyrenoids; lorica 30·8 × 26·4 μm; spines 7–9 μm long.

Habitat: In cultures of silt from location 61 (NP 16, September).

Though the typical species (see figure 269 in Deflandre 1926) could occasionally have a crown of spines around the pore, they are of variable length and in addition to the spines on the lorica. In the present variety they are more or less uniform in length and the rest of the lorica is devoid of spines except for 3 spines seen in the posterior half. The lorica is also slightly smaller. So, it is treated as a new variety.

25. *Trachelomonas horrida* Palmer

Deflandre 1926, p 85, figure 283; Huber-Pestalozzi 1955, pp 307–308, figure 579.

Lorica ellipsoid, verrucose, densely covered with robust prismatic spines which become pointed towards their extremities rather suddenly; collar depressed and with somewhat widened undulate border; collar sometimes absent; lorica 40 μm long.

The typical species not observed in the author's collections. However, Hosmani and Bharati (1983) have reported it from Karnataka. Their species measured 39·8–41·5 × 24·9–28·2 μm.

var. *moenacanthum* Skvortzov (figure 25)

Skuja 1949, p 164; Huber-Pestalozzi 1955, p 308, figure 581.

Differs from the typical species in the collar being well developed, cylindrical and with dents at the free end, and the spines on the lorica being reduced to very short prismatic ones and less dense; membrane covered with warts or verrucae as in type; lorica including collar 40–42 × 21·2 μm; collar 6–7·5 × 5–5·5 μm.

Habitat: Stray in location 14.

Huber-Pestalozzi does not give the dimensions of this variety. Skuja gives them as 37–39 (with collar) × 20–22 μm, with collar 5–7 × 5–6 μm. The Assam specimen was slightly larger.

Distribution in Indian region: Burma (Skuja 1949); Assam (!).

26. *Trachelomonas armata* (Ehr.) Stein 1878 (figure 26a)

Lemmermann 1910, p 527, figure 24 (p 517); Deflandre 1926, p 87, figures 312, 315, 319 and 320; Huber-Pestalozzi 1955, p 308, figure 582.

Lorica ellipsoid or slightly ovoid with rounded ends, anterior end frequently narrower; posterior end with a crown of well developed spines which are curved and more or less convergent, and of variable length, rarely reduced to knob-like projections or short papillae; pore with or without a ring-like thickening or sometimes with a slightly depressed collar; outer edge of pore usually denticulate; membrane smooth or with fine dense punctae, and deep yellowish brown; lorica 29·9–35·4 × 25·5–27·3 μm; spines 1–9 μm in length; chromatophores numerous, discoid and without pyrenoids.

Habitat: Stray to rare in locations 1a, 5b, 5c, 6b, 54, 63 (NP 32, September), 65, 76, 92, 100a, 136b, 137d and 142a.

The organism agreed well with the typical species measuring 29–37 × 22–29 μm with spines 1–9 μm long (see Deflandre 1926). Lemmermann gives the length of the lorica as 29–64 μm, probably including the spines.

Distribution in Indian region: Burma (Skuja 1949); Hyderabad (Zafar 1959);

Bombay (Kamat 1964, 34–37·5 × 22–24 µm); Alibag, Maharashtra (Kamat 1968); Kerala (Suxena *et al* 1973, 36–37 × 25–26 µm); Vidarbh (Kamat 1975); Assam, Orissa, Andhra Pradesh and Kerala (!).

var. *steinii* Lemm. emend. Defl. 1926 (figure 26b)

Deflandre 1926, p 88, figures 314, 316, 322–324 and 326–327; Huber-Pestalozzi 1955, p 309, figure 585; = *T. armata* var. *steinii* Lemm. 1904.

Lorica as in the typical species but often larger, with one or more crowns of spines of varying lengths and thickness around the pore; hind end with well developed spines as in the typical species or with a mixture of well developed and ill developed spines; posterior spines sometimes divergent; apical spines occasionally obtuse; apical pore with or without an annular thickening and with or without a depressed collar bearing teeth at the outer edge; lorica 40–42 × 31–32 µm (without spines).

Habitat: Stray in locations 4, 6a and 136a; rather common in location 102 and common in location 136b.

The organism agreed fairly well with Lemmermann's variety which measures according to Deflandre (1926) 37–40 × 30–33 µm without spines. Skuja (1949) gives the length of the lorica as 45 µm with spines and 39 µm without, and width 33 µm.

Distribution in Indian region: Burma (Skuja 1949, 39 × 33 µm); Bombay (Gonzalves and Joshi 1946; Kamat 1964, 47–55 × 33–36 µm); Kolhapur (Kamat 1963, 40–50 × 30–35 µm); Alibag (Kamat 1968); Vidarbh (Kamat 1975); Assam, Andhra Pradesh and Kerala (!).

forma *punctata* (Swirenko) Defl. 1926 (figure 26c)

Deflandre 1926, p 88, figure 313; Huber-Pestalozzi 1955, p 309, figure 586; = *T. armata* var. *punctata* Swirenko; = *T. armata* var. *steinii* Bourrelly 1961, p 307, Plate 7, figure 2.

Differs from var. *steinii* Lemm. in the membrane being provided with small dense punctae; lorica 30 × 26 µm.

Habitat: Stray in location 63 (NP 62, September).

The organism agreed fairly well with Swirenko's variety but was slightly smaller, Swirenko's variety measuring 43–52 × 30–35 µm and Bourrelly's 50 × 40 µm. However, both these included the spines also.

Distribution in Indian region: Orissa (!).

var. *duplex* Playfair emend. Playfair 1921

Playfair 1921, p 133, plate 7, figure 5.

Lorica ovoid with the posterior end broader and covered not compactly with fine short spine (not granulate); both anterior and posterior ends of lorica with a crown of medium-sized to fairly long spines; anterior spines more or less straight, acute (not bacillar) and about 12 in number; posterior spines also acute, recurved and about 10 in number; membrane brown; lorica 45 × 35 μm with anterior spines about 8 μm and posterior ones 24 μm.

The typical variety not observed by the author.

In his original description Playfair (1915) characterised the variety as having a granular membrane with about 12 anterior rod-like (bacillar) spines about 8 μm long and about 10 posterior pointed and slightly curved spines up to 12 μm long. Both Deflandre (1926) and Huber-Pestalozzi (1955) based their description of the variety on this description. Playfair (1921), however, had corrected his earlier description with a fresh figure and characterised the lorica as hispid with short spines and not granular, the anterior spines as acute and not bacillar, the dimensions of the lorica being retained as 45 × 35 μm, with posterior spines up to 24 μm long.

forma *jorhatensis* Philipose f. *nov.* (figures 26d, e)

Differt varietas a var. *duplex* eo quod lorica habet tantum 7–9 rectas vel paululum curvatas spinas ad finem anteriorem necnon quinque recurvatas spinas ad finem posteriorem, his aut regulariter dispositis aut paulo ad latera (3 ad alterum et 2 ad latus alterum); membranum hispidum; lorica 46–50 × 35·5–36·5 μm, cum spinis anterioribus ad 10 μm posterioribusque spinis usque ad 21·2 μm.

Habitatio: Rara in locatione 17 (mense Octobri).

Differs from var. *duplex* in the lorica having only about 7–9 straight or slightly curved spines at the anterior end and 5 recurved spines at the posterior end, the latter either regularly disposed or somewhat laterally (3 on one side and 2 on the other); membrane hispid; lorica 46–50 × 35·5–36·5 μm with anterior spines up to 10 μm and the posterior ones up to 21·2 μm.

Habitat: Rare in location 17 (October).

Distribution in Indian region: Assam (!).

var. *malabarica* Philipose var. nov. (figure 26f)

Lorica est perfecte ovoidea habensque ad finem anteriorem corymbum brevium (3·5–5·3 μm) obtusarum, rectarumque vel paulo curvatarum spinarum necnon 3–4 longissimas (24·6–26·4 μm) crassas recurvatasque spinas immixtas cum 4–5 spinis magnitudinis mediae (8·5–15 μm) cum nonnullis brevioribus (1·8–2·5 μm) acutis spinis irregulariter distributis; superficies tota loricae quoque est condensa cum brevioribus lateque separatis cuneiformibus spinis obtusis in forma vertentibus ad acutas; membranum plene fulvum; lorica 37 × 32·6 μm (spinis exclusis).

Habitatio: Aberrans in locatione 138 (mense Octobri).

Lorica perfectly ovoid and with a cluster of short (3·5–5·3 μm) obtuse, straight or slightly curved spines at the anterior end and 3–4 very long (24·6–26·4 μm) stout recurved spines interspersed with 4–5 medium-sized (8·5–15 μm) and a number of very short (1·8–2·5 μm) acute spines irregularly distributed at the posterior end; entire surface of lorica also covered with very short widely spaced conical, obtuse to acute spines; membrane deep brown; lorica 37 × 32·6 μm (excluding spines).

Habitat: Stray in location 138 (October).

Distribution in Indian region: Kerala (!).

var. *longispina* Playfair emend. Deflandre 1926

Deflandre 1926, pp 88–89, figures 324–330 and 334–335; Huber-Pestalozzi 1955, p 309, figure 587; = *T. armata* var. *longispina* Playfair 1915; 1921, p 133.

Lorica as in the typical species but more ovoid, with the posterior and anterior ends broader and provided with a variable number (usually 4–10) of long well developed spines which are somewhat curved; the entire lorica also covered with short thick conical spines which are widely placed (about 4 in 10 μm), and usually yellowish, sometimes colourless; these spines are sometimes smaller and closer at the hind end; lorica light to deep brown; dimensions of lorica without spines 43–44 × 32–34 μm with short spines 5–6 μm and posterior ones 17–24 μm (after Playfair) or 41–45 × 32–34 μm with short spines 3–6 μm and posterior ones up to 15 μm (after Deflandre).

The typical variety was not observed by the author but reported from Maharashtra by Kamat (1975) and from Karnataka by Hosmani and Bharati (1983, 41·5–44·8 × 33·2–34·9 μm).

forma *punctata* Philipose f. *nov.* (figure 26g)

Forma a var. *longispina* differens eo quod loricae integrae membranum est punctatum; dimensione (spinis exclusis) 42 × 31·7 μm, cum vere spinis brevibus usque ad 6 μm, posterioribusque usque ad 15 μm longitudinis.

Habitatio: Aberrans in locatione 136a (mense Februario).

Differs from var. *longispina* in the entire lorica membrane being punctate; dimensions (excluding spines) 42 × 31·7 μm with short spines up to 6 μm and posterior ones up to 15 μm long.

Habitat: Stray in location 136a (February).

Distribution in Indian region: Kerala (!).

var. *longa* Deflandre 1926 (figures 26h, j)

Deflandre 1926, p 89, figures 332–333 and 337; Huber-Pestalozzi 1955, p 310, figure 591.

Lorica always narrower than in the typical species with the poles sometimes slightly flattened; anterior pole with one or more crowns of small spines as in var. *steinii*; pore without collar and with a few long spines; membrane fairly thick and with fine dense punctae and sometimes with a thickening at the hind pole; lorica light brown and 39–40 × 29–30 μm.

Habitat: Rare in location 92 (April) and stray in location 152 (November).

The organism from location 152 was smaller (32·5 × 24 μm) with short spines at both ends, the posterior pole having two additional stouter ones (figure 25j) and the membrane was smooth and the lorica colourless. Probably it was a juvenile form.
Dimensions of this variety as given by Deflandre are 41–43 × 29–30·5 μm.

Distribution in Indian region: Orissa and Tamil Nadu (!).

27. *Trachelomonas dangeardiana* (Defl.) Huber-Pestalozzi 1955

Huber-Pestalozzi 1955, p 311, figure 594; = *T. dangeardiana* Defl., Bourrelly 1961, p 308, plate 6, figure 11; = *T. dangeardi* Deflandre 1926, pp 89–90, figures 295, 317 and 339–341.

Lorica usually ellipsoid, rarely ovoid, sometimes with arched sides and broadly rounded ends; posterior end with a crown of well developed pointed spines which are usually straight and divergent and of variable length; sometimes without such spines; pore with or without an annular thickening; membrane yellowish brown and densely covered with short stout rod-like spines; lorica 36–42 × 27–32 μm.
The typical species not observed by the author.

var. *glabra* (Playfair) Deflandre 1926 (figure 27a)

Deflandre 1926, p 90, figures 338, 343; Huber-Pestalozzi 1955, p 311, figure 596; = *T. armata* var. *glabra* Playfair 1915.

Lorica and the posterior spines as in the typical species, membrane otherwise completely smooth, rarely punctate; lorica 35 × 28·5 μm.

Habitat: Stray in location 76 (February).

Deflandre gives the dimensions as 34–46 × 28–34 μm (after Playfair) or 40 × 31 μm (after Deflandre).

Distribution in Indian region: Orissa (!).

var. *cylindrica* Philipose var. nov. (figure 27b)

Lorica a forma oblongata varians ad cylindratum cum 5–6 crassis magnitudinis intermediatae paulumque curvatis spinis ad finem posteriorem; superficies loricae brevibus obtusiusque in forma ad habetes spinas condensae; membranum paulo flavim coloris; fistula habet crassitudinem anularem; lorica 43 × 28 μm; fistula circa 9 μm diametri.

Habitatio: Rara locatione 106a, aberransque in locatione 136a (mense Februario).

Lorica oblong to cylindrical with 5–6 stout medium-sized somewhat curved spines at the hind end; surface of lorica covered with short obtuse to blunt spines; membrane light brown; pore with annular thickening; lorica 43 × 28 μm, apical pore 9 μm in diameter.

Habitat: Rare in location 106a and stray in location 136a (February).

The variety differs from the typical species in the oblong to cylindrical shape of the lorica and the posterior spines being somewhat curved and slightly convergent, and the small spines being not as stumpy.

Distribution in Indian region: Andhra Pradesh and Kerala (!).

28. *Trachelomonas eurystoma* Stein Sec. Playfair 1915

Deflandre 1926, pp 95–96, figures 418–419; Huber-Pestalozzi 1955, p 315, figure 611; Suxena 1979, p 123, plate 6, figure 65.

Lorica ovoid, broadly rounded; sides attenuated slightly towards the posterior pole; pore wide with a depressed collar which is undulate to emarginate and slightly retracted at the outer end; membrane light brown and with oblique to spiral striations running from right to left and made up of fine punctae or scrobicuations; lorica 26 × 19 μm with collar 1 μm high and 9 μm broad (Playfair), 26–27·5 × 19·5–20 μm with collar 1·1 × 5·5 μm (Suxena 1979).

The typical species not observed in the author's collections but reported from Himalaya (Suxena 1979).

var. *nuda* Szabados 1939 (figure 28)

Huber-Pestalozzi 1955, p 316, figure 616.

Lorica ovoid to heart-shaped, membrane smooth and light brown; collar depressed and about 1·5–2 μm high and 6 μm broad with smooth obliquely cut free end; lorica 24 × 17 μm.

Habitat: Stray to rare in locations 6 (June) and 159 (December).

The organism agreed fairly well with Szabados's variety. The collar in Szabados's variety is either 1 μm high and 3 μm broad with smooth, finely toothed or irregularly serrate margin or 3 μm high and 6 μm broad with obliquely cut collar. The organism from Assam and Vellore was more like the latter. Szabados gives the diameter of the lorica as 15–35 μm.

Distribution in Indian region: Assam and Tamil Nadu (!).

29. *Trachelomonas zorensis* Deflandre 1926 (figure 29)

Deflandre 1926, p 92, figures 391–395; Huber-Pestalozzi 1955, p 320, figure 627; Hortobágyi 1957, p 10, figures 102–104, 1959, p 510, figures 15–16.

Lorica ellipsoid with slightly arched sides and broadly rounded poles; pore without collar and with or without an annular thickening; membrane light yellowish brown and thickly covered with fine scrobiculae; chromatophores and flagellum not observed; lorica 16·5 × 14 μm.

Habitat: Stray in location 14 (February).

Deflandre gives the dimensions as 19–22 × 14–16 μm, and states that there are two chromatophores with diplo-pyrenoids and the flagellum is double the length of the body. Middelhoek (1951) also states that there are two chromatophores in his specimens measuring 22·8 × 19 μm. Hortobágyi (1957) gives the measurements as 15·7–23 × 14·2–19·8 μm with the pore 1·5–3 μm, which is more in agreement with the dimensions of the present form. Hortobágyi (1959) gives the number of chromatophores as 2 or 3. Huber-Pestalozzi (1955) gives the author of the species as Lefèvre which is obviously a mistake since Lefèvre's contributions appear to be subsequent to Deflandre's work on *Trachelomonas*.

Distribution in Indian region: Assam (!).

30. *Trachelomonas abrupta* Swir. emend. Defl. 1926

Deflandre 1926, p 93, figures 342–352, 354–361 and 364–365; Huber-Pestalozzi 1955, p 320, figure 628; = *T. australis* Playfair 1915.

Lorica ellipsoid-cylindrical with slightly arched sides, sometimes parallel in part; poles broadly rounded; pore always without a collar; membrane thickly covered with fine scrobiculae and bright to light yellowish brown; chromatophores 10 and without pyrenoids; eye-spot present; lorica 22–30 × 12–19 μm.

The typical species not observed by the author, but reported from Burma (Skuja 1949, 29 × 20 μm) and Karnataka (Hegde and Bharati 1986).

var. *minor* Deflandre 1926 (figure 30a)

Deflandre 1926, p 93, figures 371–378 and 381–388; Huber-Pestalozzi 1955, p 320, figure 629.

Lorica as in the typical species but smaller and densely punctate with the punctae sometimes indistinct; membrane fairly thick and bright yellowish brown; chromatophores about 10, discoid and without pyrenoids; lorica 22–23·1 × 9·9–11·4 μm, pore 3·5 μm in diameter; flagellum slightly longer than body.

Habitat: Stray in locations 14 (February) and 136a (February).

The organism agreed fairly well with Deflandre's variety measuring 15·5–22 × 9–12 μm.

Distribution in Indian region: Bombay (Kamat 1964, 19·5–22 × 9–9·8 μm); Assam and Kerala (!).

var. *arcuata* (Playf.) Defl. 1926 (figure 30b)

Deflandre 1926, p 93, figures 366–368; Huber-Pestalozzi 1955, p 320, figure 631; = *T. australis* var. *arcuata* Playfair 1915.

Lorica elongate-ellipsoid, not cylindrical, with the sides arched and poles broadly rounded; dimensions 28 × 21 μm, with pore 4·8 μm in diameter.

Habitat: Stray in location 13 (April).

The organism was slightly broader than Playfair's variety which measures 20–27 × 14–17 μm (after Playfair) and 30 × 18 μm (after Deflandre).

Distribution in Indian region: Assam (!).

31. *Trachelomonas granulosa* Playfair 1915 (figure 31a)

Deflandre 1926, p 95, figures 401 and 414–415; Huber-Pestalozzi 1955, p 322, figure 641; Hortobágyi 1963, pp 366–367, figure 870.

Lorica ellipsoid with the sides arched, densely crowded with small granulations or fine pearls, collar very much depressed or absent; membrane yellowish to reddish brown; internal contents not observed; lorica 19·4 × 14·1 μm with pore 3·5 μm in diameter.

Habitat: In cultures of silt from location 61 (September).

The organism agreed fairly well with Playfair's species (17–26 × 13–22 μm). Deflandre includes this species under "Species Inqirenda". According to Hortobágyi (1963) there are numerous discoid chromatophores without pyrenoids and a flagellum about 1½ times body length in this species. He gives dimensions of 17–22·8 × 14–20·3 μm for the lorica with the diameter of pore 3·5 μm.

Distribution in Indian region: Orissa (!).

var. *crenulatocollis* (Szab.) Huber-Pest. 1955

Huber-Pestalozzi 1955, p 322, figure 644A; = *Trachelomonas intermedia* var. *crenulatocollis* Szabados.

Lorica ellipsoid, deep to reddish brown, coarsely punctate or with small lens-like elevations, 20–24·7 × 16–20 μm; collar short and widened towards the outside and with a few tooth-like protuberances 1·7–2·8 μm high and 5–5·4 μm broad at the pore.

Known only from Hungary.

forma *obovata* Philipose f. *nov.* (figure 31b)

Lorica plus minusve obovata habens marginem paululum irregularem; paulo flavi est coloris; dimensione 28·2 × 17·6 μm magnitudine, cum collari 1·8 μm altitudine, 7·9 μm vero latitudine; fistula habet 4 tubera; chromatophora parvae, numerosae discoideasque aunt sine pyrenoidis; flagellum paulo longius quam corpus.

Habitatio: Aberrans in locatione 23 (mense Iunio).

Lorica more or less obovate with slightly irregular margin; light brown and 28·2 × 17·6 μm, with collar 1·8 μm high and 7·9 μm broad; pore with 4 protuberances; chromatophores small, numerous, discoid and without pyrenoids; flagellum slightly longer than body.

Habitat: Stray in location 23 (June).

Distribution in Indian region: West Bengal (!).

32. *Trachelomonas scabra* Playfair 1915 (figure 32a)

Deflandre 1926, p 98, figures 456–457 and 463–465; Huber-Pestalozzi 1955, p 323, figure 646.

Lorica ellipsoid with both poles evenly rounded or the hind end acuminate; pore with a wide depressed collar; membrane scabrous but never with regular granulations, pale to medium yellowish brown; lorica 20–23 × 15–16·7 μm; pore 3·5 μm in diameter; flagellum about 3 times body length.

Habitat: Very rare to rare in locations 2, 19, 23, 28 (April), 29 (April), 45, 46, 63 (NP 24, November), 71, 88d, 100, 117 and 157; Rather common in locations 27 (May), 109 (December) and 151 (February and July), and very common in 151 (April).

Usual dimensions of this species are 20–23 × 15–20 μm (see Deflandre 1926).

Distribution in Indian region: Maharashtra (Kamat 1968, 1975); Uttar Pradesh (Hortobágyi 1969, 18 × 14·7 μm); Assam, West Bengal, Orissa, Andhra Pradesh and Tamil Nadu (!).

var. *longicollis* Playfair 1915 (figure 32b)

Deflandre 1926, pp 98–99, figures 458–461 and 468; Huber-Pestalozzi 1955, p 323, figure 647.

Differs from the typical species in the lorica having a long collar which is straight, oblique or sometimes curved, and with the outer end broadened; lorica 24·6–26·2 × 16·7–18·1 μm, collar 5·3–6·1 × 3–4 μm.

Habitat: Rather common in location 28 (April) and stray in locations 5b (June) and 63 (NP 24, November).

Usual dimensions of this variety are 22–33 × 16–19 μm with collar 5–7 μm high and 3–5 μm broad (see Deflandre 1926).

Distribution in Indian region: Assam, West Bengal and Orissa (!).

var. *coberensis* Deflandre 1926 (figure 32c)

Deflandre 1926, p 99, figures 453–455; Huber-Pestalozzi 1955, p 324, figure 654.

Lorica subspherical, sometimes slightly attenuated towards the front, and with a cylindrical collar; membrane scabrous and light to bright yellowish brown; lorica 18·1–19·4 × 16·7–18·5 μm with collar 3·5 × 3·5 μm.

Habitat: Rare in location 28 (April, May, July); stray in location 63 (NP 24, November) and 102.

Dimensions given by Deflandre are 18–20 × 17–19 μm with collar 3–4 × 3–4 μm.

Distribution in Indian region: West Bengal, Orissa and Andhra Pradesh (!).

var. *labiata* (Teiling) Huber-Pestalozzi 1955 (figure 32d)

Huber-Pestalozzi 1955, pp 324–325, figure 655A; = *T. labiata* Teiling.

Lorica ovoid to ellipsoid, yellowish brown, surface slightly flattened and covered with coarse warts; hind end sometimes slightly twisted on one side; pore with two lip-like folds; lorica 21·2–22 × 15·8–16·7 μm, pore 5·3 μm in diameter.

Habitat: Stray in locations 5c (June) and 14 (February).

The orgenism agreed with Teiling's species (20–22 × 17–18 μm) except that it was slightly narrower.

Distribution in Indian region: Assam (!).

33. *Trachelomonas szabadosiana* Huber-Pest. 1955

Huber-Pestalozzi 1955, p 326, figure 661A; = *T. ornata* Szabados 1940 *non T. ornata* Skvortzov 1925.

Lorica ellipsoid with numerous broad-based processes on its surface, showing a net-like sculpturing; membrane rust brown; lorica 21 × 19·2 μm with pore 2 μm in diameter; flagellum twice as long as the body.

Known only from Hungary.

forma *minor* Philipose f. *nov.* (figura 33)

Lorica est minoris magnitudinis (17·6 × 14·0 μm) cumque processubus paucioribus.

Habitatio: Aberrans in Locatione 63 (NP 32, mense Octobri).

Lorica smaller (17·6 × 14·0 μm) and with fewer processes.

Habitat: Stray in location 63 (NP 32, October).

Distribution in Indian region: Orissa (!).

34. *Trachelomonas aspera* Da Cunha (figure 34)

Huber-Pestalozzi 1955, p 326, figure 662; *non T. aspera* (Skv.) Deflandre 1926, p 114, figure 618.

Lorica ellipsoid with both poles evenly rounded and light brown; surface with irregular tubercles and wrinkles giving the sides of the lorica an undulate appearance; pore with a straight collar of medium width and with smooth margin; chromatophores discoid and without pyrenoids; eye-spot prominent; flagellum 2–3 times body length; lorica 22 × 14·8 μm.

Habitat: Stray in locations 104 and 157.

The organism agreed well with Da Cunha's species measuring 20–25 × 15 μm. *T. aspera* (Skv.) Deflandre 1926 (= *T. regularis* (Lemm.) Skvortzov) is actually *Strombomonas aspera* (Skv.) Deflandre 1930, p 607, figure 128, which Deflandre considers doubtful, since it is insufficiently described.

Distribution in Indian region: Andhra Pradesh and Tamil Nadu (!).

35. *Trachelomonas planctonica* Swirenko

Deflandre 1926, p 102, figures 494–499; Huber-Pestalozzi 1955, p 328, figure 668.

Lorica subspherical with the surface strongly punctate or finely scrobiculate; pore with a robust, blunt, conical collar with irregularly dentate margin; membrane thick and dark yellowish brown; with 8–10 chromatophores; lorica 14–30 × 17–22 μm; collar 4–5 μm broad, 2·5–4 μm high.

The typical species not observed by the author.

forma *minor* Philipose *et* Radhakrishnan forma *nov.* (figure 35)

Forma a species typica differens on magnitudinem minorem, videolicet, lorica 12·3 × 8·8 μm, collare 3·2 μm latitudinis necnon 2·3 μm altitudinis.

Habitatio: Aberrans in locatione 14 (mense Februario).

Differs from the typical species in its smaller size, viz. lorica 12·3 × 8·8 μm; collar 3·2 μm broad and 2·3 μm high.

Habitat: Stray in location 14 (February).

Distribution in Indian region: Assam (!).

36. ***Trachelomonas volzii*** **Lemm. 1904**

Lemmermann 1910, p 528; 1913, p 151, figure 281; Deflandre 1926, pp 105–106, figure 534; Huber-Pestalozzi 1955, p 333, figure 689.

Lorica ovoid to somewhat pear-shaped; posterior end rounded, sides slightly arched and attenuated towards the front; pore with a cylindrical collar surrounded by a characteristic conical membrane which binds the collar wall with the wall of the lorica at the pore margin; membrane with fairly dense granulations; lorica 32 × 15 μm with collar 4 μm high.

The typical species known only from Sumatra, Java and France.

var. ***cylindracea*** **Playfair 1915 (figures 36a, b)**

Deflandre 1926, p 106, figures 538–539; Huber-Pestalozzi 1955, p 334, figure 695.

Lorica more or less cylindrical with the sides parallel; posterior end broadly rounded; anterior end conical with the sides converging towards the front; collar straight, robust and provided with a basal annular ring; membrane smooth and light yellow; lorica 32–36 × 15–16·7 μm; collar 5·3 μm high and 3·5 μm broad.

Habitat: Rare in locations 92 and 136a; rather common in location 102.

The organism agreed fairly well with Playfair's variety measuring 34–38 × 16 μm with collar 4–5 μm high and 4 μm broad. Prowse (1958, p 183, figures 6 j, k) gives the dimensions as 32–42 × 16–17 μm.

Distribution in Indian region: Bombay (Kamat 1964, 34–38 × 14–16 μm, collar 4–5 × 3·5–4 μm); Vidarbh (Kamat 1975); Orissa, Andhra Pradesh and Kerala (!).

37. ***Trachelomonas dubia*** **Swir. emend. Defl. 1926**

Deflandre 1926, pp 106–107, figures 544 and 555–561; Huber-Pestalozzi 1955, p 334, figure 696.

Lorica ellipsoid-cylindrical with the sides parallel up to about 1/3 of its length, sometimes slightly undulate or slightly convergent towards the anterior end; front end always broadly rounded to slightly flattened; hind end more or less rounded, sometimes slightly conical or distinctly pointed; pore with or without a ring-like thickening and always with a straight cylindrical fairly high collar which is cut straight; ring-like thickening of pore often markedly developed making it narrower than the collar; membrane smooth and yellowish to dark reddish brown; with 7–10 large chromatophores; flagellum 2–2½ times body length; lorica 22–26 × 11–14 μm.

Though both Deflandre and Huber-Pestalozzi state there are no pyrenoids in the

Chromatophores, Hortobágyi (1963, p 365, Tafel 66, figure 662) has recorded pyrenoids and rod-shaped paramylum bodies in this species.

The typical species not observed in the author's collections.

var. *lata* Deflandre 1926 (figure 37)

Deflandre 1926, p 107, figure 792; Huber-Pestalozzi 1955, p 335, figure 699; = *T. euchlora* (Ehr.) Lemm. in Skuja 1926.

Deffers from the typical species in the greater breadth of the lorica in relation to its length; internal contents not observed; lorica 25 × 15·8 μm with collar 2·5 × 3 μm.

Habitat: Stray in location 28 (April).

Dimensions given by Deflandre are 24–27 × 16 μm.

Distribution in Indian region: Maharashtra (Kamat 1975); West Bengal (!).

38. *Trachelomonas hexangulata* (Swirenko) Playfair (figure 38)

Deflandre 1926, p 107, figures 545–551; Huber-Pestalozzi 1955, p 335, figure 702; = *T. ampullula* Playfair 1915.

Lorica nearly 6-sided in front view with rounded angles and the sides in the median region almost parallel, and front and hind ends conical; hind pole more or less truncate or sometimes broadly rounded; pore with or without a raised ring-like thickening which makes the pore narrower than the collar; collar cylindrical, straight or sometimes with a membrane cone joining the collar wall with the upper margin of the lorica; membrane smooth and light yellowish; lorica 25 × 10·7 μm with collar 2 × 3 μm.

Habitat: Rather common in location 109 (December); stray in location 137 (September).

The usual range of dimensions of this species is 24–34 × 10–16 μm with collar 2–5 μm high and 3–5 μm broad (see Deflandre 1926).

Distribution in Indian region: Bombay (Kamat 1964, 20–25 × 8–10 μm); Andhra Pradesh and Kerala (!).

39. *Trachelomonas bulla* Stein emend. Defl. 1926 (figure 39)

Deflandre 1926, p 110, figures 592 and 571–578; Huber-Pestalozzi 1955, p 340, figure 719.

Lorica elongate-ellipsoid to ovoid with the hind end sometimes attenuated, irregularly ornamented with very short spines which are often reduced as pointed to obtuse denticulations; membrane sometimes rugged; pore small and topped by a broad conical, punctate, rugged or smooth collar which sometimes appears as a ontinuation of the sides of the lorica and with the free end straight or slightly blique and provided with irregular denticulations; lorica 42 × 22 µm; collar 5 × 4·5–6 µm.

abitat: Stray in locations 80 (December) and 100a (February).

Dimensions given by Deflandre and Huber-Pestalozzi are 30–39 µm (without ollar) and 36–47–50 µm (with collar) × 20–24 µm; collar 5·5–8 µm high, 7–11 µm base and 4–6·5 µm at the opening.

istribution in Indian region: Maharashtra (Kamat 1868); Karnataka (Hosmani nd Bharati 1983, 30–30·6 × 23–23·4 µm); Orissa (!).

). ***Trachelomonas playfairi*** **Defl. 1924 (figure 40)**

eflandre 1924, p 1125, plate 10, figures 14–15; 1926, pp 110–111, figures 580–582; uber-Pestalozzi 1955, p 341, figure 721; Rino 1972, p 163, plate 8, figures 2–3; *T. euchlora* (Ehr.) Lemm. forma Playfair 1915.

orica broadly ellipsoid or sometimes indistinctly rectangular with poles broadly ounded; pore constantly with a curved collar which is as broad as long and niformly broad or slightly narrowed towards the free end which is cut more or less gularly; membrane smooth and yellowish brown; chromatophores numerous and ith projecting double-sheathed pyrenoids; lorica 20–22·5 × 15–16 µm; collar 3 × 3–5 µm; eye-spot discoid.

abitat: In cultures of silt from location 61 (NP 16, September), and in locations 76 ebruary), 93 (November) and 103a (December).

It agreed fairly well with Deflandre's species measuring 19–23 × 16–18·5 µm with ollar 3–3·5 × 3·5 µm. Rino (1972) gives the dimensions as 22–26 × 18–23 µm with ollar 3·5 µm.

istribution in Indian region: Maharashtra (Kamat 1975). Orissa and Andhra radesh (!).

r. ***hyalina*** **(Skvortzov) Hortobágyi 1969 (figure 40c)**

ortobágyi 1969, p 33, plate 5, figure 74; = *T. similis* var. *hyalina* Skvortzov 1937, 80, plate 10, figure 27; Huber-Pestalozzi 1955, p 342, figure 723; Prowse 1958, p 3, figure 6g.

orica irregularly ellipsoid, hyaline, brown, smooth and not punctate; collar tubular, blique and with smooth wall; lorica 22·5 × 15 µm with collar 3 × 2·5–2·8 µm; with veral discoid chromatophores having double-sheathed pyrenoids; eye-spot streakke.

abitat: Stray in locations 6 (June) and 151 (April).

It compared well with Skvortzov's variety measuring 18·7–23 × 15–15·5 µm with collar 2–2·5 μm and Hortobágyi's variety measuring 18·7–25 × 15·5–18 μm with collar 2–3 μm high and pore 2–4 μm.

Distribution in Indian region: Burma (Skvortzov 1937, see above); Uttar Pradesh (Hortobágyi 1969); Assam and Tamil Nadu (!).

41. *Trachelomonas similis* Stokes 1888 (figures 41a, b)

Deflandre 1926, p 111, figures 584 and 586–588; Skuja 1949, p 165, Tafel 36, figure 34; Huber-Pestalozzi 1955, p 342, figure 722; Rino 1972, p 164, plate 8, figure 4.

Lorica usually ellipsoid but sometimes ovoid with the poles rounded and more or less regularly punctate (rarely irregularly scrobiculate); pore with a collar which is always bent and with the free end irregularly dentate; membrane yellowish brown; chromatophores numerous and with double-sheathed pyrenoids; eye-spot an elongated disc; lorica 22·4–25·8 × 15–17·6 μm, collar 3–3·5 × 4·2–4·8 μm.

Habitat: Very rare to rare in locations 6 (June), 19 (April), 27 (May), 28 (April), 29 (February), 49 (December), 64 (April), 78 (January), 151 (January-April and October-December) and in cultures of silt from location 61 (NP 16, September).

The individuals from most locations agreed well with the typical species, measuring 22–40 × 14–23 μm with collar 3–3·5 × 4·5–5·5 μm, but in those from locations 29 and 151 the membrane was irregularly granulate to scrobiculate (see figure 41b). Rino also shows the lorica of *T. similis* as irregularly scrobiculate.

Distribution in Indian region: Madras (Philipose 1940, 22·5–25·8 × 15–16·3 μm); Burma (Skuja 1949, 26 × 14 μm, with neck 2·8 μm, pore 4 μm), Maharashtra (Kamat 1975), Assam, West Bengal and Orissa (!).

42. *Trachelomonas atrata* (Skv.) Deflandre

Huber-Pestalozzi 1955, p 343, figure 726; = *T. crebea* var. *atrata* Skv. 1925; = *T. troitzkajae* Skv. var. *planctonica* Skv. 1925.

Lorica ellipsoid with the posterior half having rows of somewhat distant hemispherical granulations; collar straight or bent and nearly as high as broad; membrane brown; chromatophores numerous; lorica 26–33 × 16–24 μm; collar 4–5·7 μm.

Known only from Manchuria.

var. *pustulosa* Conrad 1952 (figure 42)

Huber-Pestalozzi 1955, p 343, figure 727; = *T. decorata* (Skv.) Defl. var. *pustulosa* Conrad 1952.

Lorica ellipsoid with the collar cylindrical and always bent and the pore straight and smooth; anterior half of lorica completely smooth or with small scattered pearl-like granulations; posterior half with larger pearls or with numerous papillae which are joined in groups of 2–4 pearls; lorica 16·7 × 13·5–14 μm, collar 3·5–4·4 × 2–2·5 μm; chromatophore, paramylum and flagellum (recorded by Conrad) not observed.

Habitat: Stray in locations 29 (May) and 102 (December).

Conrad's variety measured 13–16 × 10·5–13 μm with collar 2–4 × 2 μm.

Distribution in Indian region: West Bengal and Andhra Pradesh (!).

43. *Trachelomonas gregusii* Hortobágyi (figure 43)

Huber-Pestalozzi 1955, p 344, figure 729.

Lorica ellipsoid, yellowish brown and with ridge-like elevations which are seldom straight but mostly bent differently, and uniformly thick or only thick in places, of varying length and irregularly divided; when numerous, the ridges are thinner and flatter, when fewer more pronounced, thicker and more elevated; pore with a bent obliquely cut smooth collar narrowing towards the tip; chromatophores not observed; lorica 17·6 × 15 μm; collar 2·5 × 2·5–3 μm.

Habitat: Stray in location 2 (June), 5b (June) and 6 (June).

The organism agreed with Hortobágyi's species measuring 16·5–20 × 14–16·5 μm with collar 2·5–3 × 2·5–3 μm.

Distribution in Indian region: Assam (!).

44. *Trachelomonas pseudocaudata* Defl. 1926

Deflandre 1926, pp 117–118, figures 651–654; Huber-Pestalozzi 1955, p 351, figure 755.

Lorica regularly ellipsoid and covered densely with stumpy and obtuse spines; pore with annular thickening and with a distinct straight cylindrical collar which is slightly widened towards the aperture and is denticulate; posterior part with a short hollow more or less pointed or obtuse tail which is forked and sometimes truncate and provided with 3–4 small spines; chromatophores numerous (about 8–10), discoid-polyhedral and without pyrenoids; total length of lorica 41–43·5 μm with breadth 22–23 μm, collar 5–6 μm long and 5–5·5 μm broad at base; tail 3·5–6·5 μm long.

The typical species known only from France and Switzerland.

var. *elongata* Philipose var. *nov.* (figure 44)

Varietas a species typica differens eo quod lorica est longior (54–55·5 μm) necnon angustior (18·0–18·4 μm), habetque collare 5·4–6·6 μm longitudinis et 4·8–5·2 μm latitudinis, fistulamque 5·2–6·2 μm latitudinis; caudam 8·3–9·4 μm longitudinis, et 4·2 μm latitudinis ad radicem necnon 2·1 μm ad apicem; denticulationibus fistulae etiam clare signatis; chromatophora circa 12 necnon discoideae-polyhedrales sine pyrenoidis sicut in specie typica.

Habitatio: Rara in locatione 136a (mense Februario).

Differs from the typical species in the lorica being longer (54–55·5 μm) and narrower (18–18·4 μm) with the collar 5·4–6·6 μm long and 4·8–5·2 μm broad, pore 5·2–6·2 μm wide; tail 8·3–9·4 μm long, 4·2 μm broad at base and 2·1 μm towards the tip; denticulations of the pore also more marked; chromatophores about 12 and discoid-polyhedral without pyrenoids as in the typical species.

Habitat: Rare in location 136a (February).

Distribution in Indian region: Kerala (!).

45. *Trachelomonas bernardinensis* Vischer emend. Defl. 1926 (figure 45)

Deflandre 1926, pp 118–119, figures 655–684 and 693; Huber-Pestalozzi 1955, p 352, figure 760; including var. *granulosa* Chodat.

Lorica elongate-ellipsoid with the sides usually regularly arched but sometimes slightly attenuated towards the anterior end; pore rarely with an annular thickening and provided with a wide collar which is very variable (cylindrical, subcylindrical, stumpy and conical or markedly widened at the rim) and always cut straight and almost smooth, serrate to dentate or with fine pointed spines of varying length; posterior end with a short hollow smooth tail which is also variable and is either pointed or stumpy and often open at the end; sometimes the tail is reduced to a knob; membrane deep brown and densely scrobiculate; chromatophores, stigma and flagellum not observed; cell filled with numerous discoid paramylum; lorica 37·8 × 16–17 μm.

Habitat: Stray in locations 6 (June), 35 (July) and 92 (April).

Usual dimensions of this species range from 34–43 μm (including collar) × 18–22 μm with collar 2–6 μm high and 5·5–7·7 μm broad at base and 5·5–12 μm at the opening; tail 1–3·5 μm long (see Deflandre 1926).

Distribution in Indian region: Maharashtra (Kamat 1975); Assam, West Bengal and Orissa (!).

46. *Trachelomonas helvetica* Lemm. emend. Defl. 1926

Deflandre 1926, p 121, figures 620 and 630; Huber-Pestalozzi 1955, p 356, figure 770; = *T. helvetica* Lemm. 1910, p 529; = *t. conica* var. *caudata* Playfair 1915.

Lorica unequally ellipsoid to ovoid, anterior pole rounded; sides attenuated towards the posterior end into a short truncate tail; pore large and without collar, and ornamented with some spines; entire membrane densely covered with pointed spines; lorica 38–42 × 17·5–20 μm, pore 5 μm wide.

The typical species is known only from Switzerland, Poland, Sweden and Australia.

var. *elongata* Bourrelly 1961 (figure 46a)

Bourrelly 1961, p 308, Plate 6, figure 4.

Differs from the typical species in the lorica being more elongated and narrower; lorica (including tail) 43·8 × 15·6 μm; pore 5·3 μm wide; tail 7·9 μm long.

Habitat: Stray in location 92 (April).

It agreed with Bourrelly's variety measuring 40–55 × 15–15·5 μm.

Distribution in Indian region: Orissa (!).

var. *cucurbita* (Skv.) Huber-Pest. 1955 (figure 46b)

Huber-Pestalozzi 1955, p 356, figure 771; = *T. cucurbita* Skvortzov; = *T. helvetica* var. *hispida* Skvortzov.

Differs from the typical species in its slightly smaller size and in the regularly rounded hind end; lorica (including tail) 33·5 × 15 μm; pore 3·5–4·4 μm.

Habitat: Stray in location 76 (February).

The variety usually measures 30–35 × 14·5–15·7 μm with the pore 4·2 μm wide.

Distribution in Indian region: Orissa (!).

47. *Trachelomonas nadsonii* Skvortzov 1925

Huber-Pestalozzi 1955, p 358, figure 774.

Lorica spindle-shaped with regularly arched sides and regular but not closely arranged spines; anterior end narrowed into a long broad neck with widened dentate mouth; posterior end narrowed into a tail; ornamentation of neck and tail as in the rest of the lorica; lorica smooth between the spines, 66·5 × 22·5 μm with neck 9·2 μm and tail 14·8 μm long; chromatophores numerous and discoid; paramylum bodies spherical.

Known only from Manchuria.

var. *indica* Skvortzov 1937

Skvortzov 1937, p 85, plate 12, figures 9–10.

Lorica spindle-shaped, 50–55 μm long, 17 μm broad with neck 8–8·5 μm long and 5–5·2 μm broad; tail more or less pointed; lorica membrane covered with irregular little broad spines or punctae; neck enlarged and serrated at the pore; differs from the typical species in the broader neck and punctate membrane.

Distribution in Indian region: Burma (Skvortzov 1937).

forma *kausalyagangensis* Philipose f. *nov.* (figurae 47a, b)

Forma a var. indica Skv. dicta differens eo quod habet caudam crassius paulo bifurcatumque ad apicem; lorica quoque est paulum longior (59·0–61·6 μm), 17·6 μm lat., habens collum 7·9–8·8 × 5·3 μm, caudam 8·8–10·6 μm; fistula 6·3 μm diametri.

Habitatio: Rara in locatione 92 (mense Aprili).

Differs from var. *indica* Skvortzov in its stouter tail which is slightly bifurcate at the tip. The lorica is also slightly longer (59–61·6 μm), with breadth 17·6 μm, neck 7·9–8·8 × 5·3 μm and tail 8·8–10·6 μm; pore 6·3 μm in diameter.

Habitat: Rare in location 92 (April).

Distribution in Indian region: Orissa (!).

Other taxa of *Trachelomonas* reported from the Indian region are as follows:

(1) *Trachelomonas abrupta* Swir. emend. Defl. 1926; Burma (Skuja 1949, p 164); Karnataka (Hegde and Bharati 1986, p 63).
(2) *T. anguste-ovata* Conrad; Aurangabad (Ashtekar 1982, p 157).
(3) *T. armata* (Ehr.) Stein var. *indica* Skvortzov 1937, p 83, from Rangoon. This variety with absence of long spines from its hind end and with a ring round the pore seems doubtful.
(4) *T. armata* (Ehr.) Stein var. *ovata* Swirenko; Rangoon (Skvortzov 1937, p 83).
(5) *T. atomaria* Skvortzov var. *elegans* Skvortzov; Aurangabad (Ashtekar 1982, p 157).
(6) *T. australica* (Playf.) Defl. 1926; Burma (Skuja 1949, p 164); Himalaya (Suxena 1979, p 121).
(7) *T. australica* (Playf.) Defl. var. *rectangularis* forma; Himalaya (Suxena 1979, p 123).
(8) *T. bacillifera* Playf. 1915; Vidarbh (Kamat 1975, p 467).
(9) *T. bernardii* Wolosz. f. *major* Defl. 1926; Kodaikanal (Suxena 1983, p 67).
(10) *T. bulla* Stein emend. Defl. 1926 var. *ampullaeformis* Suxena 1983; Kodaikanal (Suxena 1983, p 67). This variety is almost like *T. volzii* Lemm. var. *cylindracea* Playfair.
(11) *T. caudata* (Ehr.) Stein; Kodaikanal (Suxena 1983, p 67).
(12) *T. cervicula* Stokes (= *T. volvocina* Ehr. var. *cervicula* (Stokes) Lemm.); Burma (Skuja 1949, p 165).
(13) *T. charkowiensis* Swirenko; Karnataka (Bongale and Bharati 1980, p 104; Hosmani and Bharati 1983, p 134; Hegde and Bharati 1986, p 63).
(14) *T. conica* Playfair 1915; Vidarbh (Kamat 1975, p 467).
(15) *T. conica* Playfair var. *indica* Skvortzov 1937; Rangoon (Skvortzov 1937, p 79).
(16) *T. cordata* (Drez.) Defl. f. *minor* Defl. 1926; Kolhapur (Kamat 1963, p 266).
(17) *T. curta* Da Cunha et Defl. var. *subbernardii* Bourrelly; Aurangabad (Ashtekar 1982, p 157).
(18) *T. cylindrica* Ehr. Sec. Playfair var. *punctata* Skvortzov 1937; Rangoon (Skvortzov 1937, p 84).
(19) *T. desikacharyii* Kamat 1963; Kolhapur (Kamat 1963, p 266); Rajasthan (Kamat 1967, p 52); Simla (Kamat 1968a, p 275).
(20) *T. dybowskii* Drez.; Bombay (Kamat 1964, p 12); Rajasthan (Kamat 1967, p 52); Simla (Kamat 1968a, p 275); Vidarbh (Kamat 1975, p 467); Nagpur (Kamat and

Freitas 1976, p 122); Aurangabad (Ashtekar 1982, p 157).

(21) *T. eurystoma* (Stein) Playf. 1915; Himalaya (Suxena 1979, p 125).

(22) *T. globularis* (Awer.) Lemm.; Simla (Kamat 1968a, p 275); Kashmir (Compère 1983, p 143).

(23) *T. globularis* (Awer.) Lemm. var. *boyeri* (Palmer) Conrad; Vidarbh (Kamat 1975, p 467).

(24) *T. globularis* (Awer.) Lemm. var. *punctata* Skvortzov; Aurangabad (Ashtekar 1982, p 157).

(25) *T. godwardii* Prasad *et* Chaudhury 1976; Banaras (Prasad *et* Chaudhury 1976, p 75).

(26) *T. granulata* Swir. emend. Defl. 1926; Uttar Pradesh (Hortobágyi 1969, p 32).

(27) *T. hispida* (Perty) Stein var. *caudata* Lemm.; Burma (Skuja 1949, p 164).

(28) *T. hispida* (Perty) Stein var. *coronata-punctata* Skvortzov 1937; Rangoon (Skvortzov 1937, p 81).

(29) *T. hispida* (Perty) Stein var. *duplex* Defl. 1926; Burma (Skuja 1949, p 164).

(30) *T. hispida* (Perty) Stein f. *minor* Bourrelly; Kolhapur (Kamat 1963, p 266).

(31) *T. horrida* Palmer; Karnataka (Hosmani and Bharati 1983, p 134).

(32) *T. horrida* Palmer var. *crenulatocollis* Skvortzov; Bombay (Gonzalves and Joshi 1946, p 176).

(33) *T. incertissima* Defl. 1926; Vidarbh (Kamat 1975, p 467).

(34) *T. irregularis* Swirenko; Uttar Pradesh (Hortobágyi 1969, p 32).

(35) *T. kelloggii* Skvortzov var. *effigurata* Skvortzov 1937; Rangoon (Skvortzov 1937, p 81).

(36) *T. klebsii* (Klebs) Defl. 1926; Ahmedabad (Kamat 1962, p 19). Bombay (Kamat 1964, p 12); Alibag (Kamat 1968, p 98); Vidarbh (Kamat 1975, p 467).

(37) *T. lacustris* Drez. 1925; Vidarbh (Kamat 1975, p 467).

(38) *T. lacustris* Drez. var. *ovalis* Drez. emend Defl. 1926; Kodaikanal (Suxena 1983, p 70).

(39) *T. lefevrei* Defl. 1926; Ahmedabad (Kamat 1962, p 19).

(40) *T. mammillosa* Prescott; Karnataka (Bongale and Bharati 1980, p 104).

(41) *T. mangini* Defl. 1926; Vidarbh (Kamat 1975, p 468).

(42) *T. mucosa* Swirenko var. *hyalina* Skvortzov; Vidarbh (Kamat 1975, p 468).

(43) *T. nadsonii* Skvortzov var. *indica* Skvortzov 1937; Rangoon (Skvortzov 1937, p 85).

(44) *T. oblonga* Lemm. var. *attenuata* Playfair 1915; Nagpur (Kamat and Freitas 1976, p 123).

(45) *T. oblonga* Lemm. var. *major* Kamat 1964; Bombay (Kamat 1964, p 12); Vidarbh (Kamat 1975, p 468): Nagpur (Kamat and Freitas 1976, p 123).

(46) *T. oblonga* Lemm. var. *truncata* Lemm.; Alibag (Kamat 1968, p 98); Vidarbh (Kamat 1975, p 468).

(47) *T. obtusa* Palmer; Vidarbh (Kamat 1975, p 468).

(48) *T. ornata* Daday var. *maharastrensis* Kamat 1963; Kolhapur (Kamat 1963, p 266).

(49) *T. ornata* Daday var. *maius* Kamat 1963; Kolhapur (Kamat 1963, p 266).

(50) *T. ovalis* Daday var. *maharastrensis* Kamat 1963; Kolhapur (Kamat 1963, p 267).

(51) *T. perforata* (Awer). Defl. 1926; Hyderabad (Suxena 1955, p 444, Zafar 1959, p 558); Vidarbh (Kamat 1975, p 468).

(52) *T. piscatoris* (Fischer) Stokes; Vidarbh (Kamat 1975, p 468).

(53) *T. pulcherrima* Playfair 1915; Burma (Skuja 1949, p 165); Kolhapur (Kamat 1963, p 267); Kandesh (Barhate and Tarar 1985, p 185).

(54) *T. pulcherrima* Playf. var. *minor* Playf.; Vidarbh (Kamat 1975, p 468).

(55) *T. raciborskii* Wolosz.; Vidarbh (Kamat 1975, p 468).

(56) *T. richmondiae* (Playf.) Defl. 1926 var. *indica* Skvortzov 1937; Rangoon (Skvortzov 1937, p 78).

(57) *T. robusta* Swir. emend. Defl. 1926; Vidarbh (Kamat 1975, p 468); Karnataka (Bongale and Bharati 1980, p 104; Hosmani and Bharati 1983, p 134); Aurangabad (Ashtekar 1982, p 157).

(58) *T. scabra* Playf. var. *ovata* Playf. 1915; Alibag (Kamat 1968, p 98); Vidarbh (Kamat 1975, p 468).

(59) *T. similis* Stokes var. *indica* Skvortzov 1937; Rangoon (Skvortzov 1937, p 80).

(60) *T. stokesi* Drez. emend. Defl. 1926; Rangoon (Skvortzov 1937, p 80).

(61) *T. stokesiana* Palmer var. *safeeuddinii* Suxena 1979; Himalaya (Suxena 1979, p 123).

(62) *T. stokesiana* Palmer var. *torquata* (Conrad) Huber-Pest. 1955; Himalaya (Suxena 1979, p 123).

(63) *T. subverrucosa* Defl. 1926; Simla (Kamat 1968a, p 275).

(64) *T. superba* (Swir.) Defl. var. *duplex* Defl. 1926; Karnataka (Hosmani and Bharati 1983, p 134).

(65) *T. superba* (Swir.) Defl. var. *swirenkiana* Defl. 1926; Karnataka (Hegde and Bharati 1986, p 63).

(66) *T. swirenkoi* Skvortzov var. *polonica* (Skv.) Skv. 1937; Rangoon (Skvortzov 1937, p 85).

(67) *T. sydneyensis* Playf. 1915; Kolhapur (Kamat 1963, p 267); Vidarbh (Kamat 1975, p 468).

(68) *T. teres* Maskell; Aurangabad (Ashtekar 1982, p 157).

(69) *T. vermiculosa* Palmer; Kodaikanal (Suxena 1983, p 67).

(70) *T. verrucosa* Stokes 1887; Hyderabad (Suxena 1955, p 444).

(71) *T. verrucosa* Stokes var. *granulosa* (Playf.) Conrad; Burma (Skuja 1949, p 165- as *T. volvocina* Ehr. var. *granulosa* Playf.): Himalaya (Subba Raju and Suxena 1979, p 156).

(72) *T. volvocina* Ehr. var. *depressa* Conrad; Ahmedabad (Kamat 1962, p 20).

(73) *T. volvocina* Ehr. var. *derephora* Conrad; Rangoon (Skvortzov 1937, p 76); Hyderabad (Suxena 1955, p 444; Zafar 1959, p 558); Bombay (Kamat 1964, p 13); Vidarbh (Kamat 1975, p 468).

(74) *T. volvocina* Ehr. var. *minuta* Fritsch; Vidarbh (Kamat 1975, p 468).

(75) *T. volvocina* Ehr. var. *papillata* Lemm; Aurangabad (Ashtekar 1982, p 157).

(76) *T. volvocina* Ehr. var. *papillato-punctata* Skvortzov; Aurangabad (Ashtekar 1982, p 157).

(77) *T. volvocina* Ehr. var. *punctata* Playf. 1915; Alibag (Kamat 1968, p 98); Aurangabad (Ashtekar 1982, p 157).

(78) *T. volvocinopsis* Playf. var. *coronata* (Skv.) Bourrelly 1952; = *T. indica* Skv. var. *coronata* Skv. 1937; Rangoon (Skvortzov 1937, p 78).

(79) *T. volvocinopsis* Playf. var. *khannae* (Skv.) Bourrelly 1952 = *T. indica* Skv. var. *khannae* Skv. 1937; Rangoon (Skvortzov 1937, p 77).

(80) *T. volvocinopsis* Playf. var. *punctata* (Skv.) Bourrelly 1952; = *T. indica* var. *punctata* Skv. 1937; Rangoon (Skvortzov 1937, p 77).

(81) *T. volvocinopsis* Playf. var. *tubigera* (Skv.) Bourrelly 1952; = *T. indica* Skv. var. *tubigera* Skv. 1937; Rangoon (Skvortzov 1937, p 77).
(82) *T. volzii* Lemm. var. *australis* Playf. Burma (Skuja 1949, p 165).
(83) *T. volzii* Lemm. var. *pellucida* Playf. 1915; Vidarbh (Kamat 1975, p 468).
(84) *T. wermeli* Skvortzov; Vidarbh (Kamat 1975, p 468).
(85) *T. woycickii* Kocz.; Alibag (Kamat 1968, p 98).
(86) *T. woycickii* Kocz. var. *bombayensis* Kamat 1964; Bombay (Kamat 1964, p 13); Vidarbh (Kamat 1975, p 468).
(87) *T. woycickii* Kocz. var. *pusilla* Skvortzov 1925; Vidarbh (Kamat 1975, p 468); Aurangabad (Ashtekar 1982, p 157).

Trachelomonas kashmiri Bhatia (1930, p 363, figure 2) with ovate lorica bluntly pointed posteriorly, with a deeply crenulate funnel anteriorly, and with dimensions of 103 × 66 μm, and stated to resemble *T. eurystoma* Stein appears doubtful. So is the case with *T. drezepolski* Skvortzov *non* Conrad var. *indica* Skvortzov 1937, p 79 and *T. drezepolski* Skvortzov *non* Conrad var. *hispida* Skvortzov 1925; 1937, p 79. *T. paludosa* Skvortzov 1917; 1937, p 79 appears to be a *T. volzii* Lemm. 1904; *T. subglobosa* Skv. 1925 *non* Playfair emend. Defl. 1926 (See Skvortzov 1937, p 78, plate 10, figure 20) also appears doubtful.

Genus *STROMBOMONAS* Deflandre 1930

Cell as in *Euglena*, free swimming and with a single flagellum, within a rigid envelope (lorica) which is narrowed at the front end into a rather wide and more or less long neck; without clear demarcation between lorica and collar; lorica mostly narrowed towards hind end into a long point or rarely rounded; wall of lorica usually thin, very rarely thick, smooth or wrinkled, seldom with ornamentation like punctae, tubercles, perforations, spines or umbilicas as in *Trachelomonas*, but sometimes striated or ridged; thickness of lorica mostly due to deposits of iron, the lorica appearing as reddish brown or brown; the deposits in various forms like granulations or reticulations.

Chromatophores regularly numerous, very rarely two, disc-like or polygonal; pyrenoids absent in most instances; paramylum usually present, of different shapes and size and distributed all over the cell; eye-spot fairly large; flagellum as long as the lorica or shorter; vacuolar system as in other Euglenineae; protoplast joined to the lorica in young cells or separated in older cells and in some species.

Mostly in freshwater, rarely in brackishwater.

Key to the Indian species described

I. Lorica in cross section orbicular . . . Section I. *Rotundatae* Conrad
  (A) Lorica smooth and without folds, grooves or wrinkles . . . . . . Subsection 1. *Ergatae* Conrad
    (1) Lorica sack-like and without tail . . . Group Saccatae
      (a) Lorica hexagonal; hind end acute; neck absent; membrane very thick and verrucose; 37 × 21 μm. . . 48. *S. hexagonalis* sp. *nov.*
      (b) Lorica flask-shaped and ovoid to spherical; neck short; membrane smooth; 52–65 × 41·6–44·6 μm . . . 49. *S. laganaeformis*
      (c) Lorica elongate-roundish, ellipsoid or ovoid, obovoid or trapezeoid; membrane with fine or coarse tubercles . . . . 50. *S. verrucosa*

(i) Lorica broadly cylindrical or ovoid; neck straight or oblique; 25–35 × 10–25 μm . . . . . . var. *conspersa*

(ii) Lorica irregularly obovate, neck short and broad; cut straight or obliquely; 33·8–37·5 × 11·3–23·6 μm . . . . . . var. *obovata* var. *nov.*

(iii) Lorica ovoid with short broad obliquely cut neck; wall thick and with papillae; 32 × (18 –) 21·6 μm . . . . . . var. *borystheniensis*

(iv) Front end of lorica markedly narrowed; neck with end cut straight 28 × 16 μm . . . . . . var. *claviformis*

Smaller form with irregular tubercles; 21 × 10·6 μm . . . forma *minor* f. *nov.*

(2) Lorica variable in shape and with tail . . . Group Caudatae

(a) Lorica irregularly trapezeoid, narrowed in front and broadened behind; membrane with tubercles; pore irregularly emarginate; 20·5–50 × 10–28·8 μm . . . . . . 50. *S. verrucosa*

(i) Lorica usually small with base often slightly arched and with very short tail . . . . . . var. *genuina*

(ii) Lorica with neck cut straight or obliquely, and often with scalloped free end; tail short and pointed; 37–50 × 20–27 μm . . . . . . var. *zmiewika*

(b) Lorica nearly cylindrical and vase-like to subhexagonal

(i) Lorica vase-like and smooth; sides arched convexly; neck broadened at end; hind end with a pointed tail; 38–57 × 22–28 μm . . . . . . 51. *S. urceolata*

Lorica with sides arched and with short neck markedly widened at end; (58·2 –) 60 × (29 –) 30 μm . . . . . . var. *elegans*

(ii) Lorica vase-like and subhexagonal with sides straight; neck markedly broadened at end, and with emarginate margin; hind end conical and ending in a point; 27·5 × 10·5 μm . . . 52. *S. vaseformis* sp. *nov.*

(iii) Lorica subhexagonal with sides straight or concave; membrane wrinkled; tail pointed; 38–57 × 19·4 – 22–26 μm . . . . 53. *S. girardiana*

(c) Lorica nearly spherical with neck of variable length and pointed tail; 20–28 μm in diameter . . . . . . 54. *S. praeliaris*

(d) Lorica ellipsoid to spindle-shaped

(i) Lorica like a spinning top with front end broader; 34 × 18 μm . . . . 55. *S. napiformis*

Lorica more oval with short widening neck and slightly longer tail; (34 –) 48–53 × 24–25 (– 27) μm . . . . . . var. *brevicollis*

(ii) Lorica broadly ellipsoid with front and hind ends about the same width

($^{+}$) Membrane smooth; tail short and pointed; 34–37 × 22 μm . . . . . . 56. *S. ovalis*

Lorica smaller; 23 × 14 μm . . . forma *minor* f. *nov.*

($^{++}$) Membrane rugged; pore with an uniformly scalloped margin; tail short and stumpy; 41·4–43 × 23·7–24·3 μm. . . . . 57. *S. madraspatensis* sp. *nov.*

(iii) Lorica spindle-shaped and slightly elongated; collar short but

relatively long; 25–38 × 12–17 μm . . . 58. *S. fluviatilis*
- ($^+$) Lorica larger than in the typical species; 60–68·5 μm . . . . . . var. *levis*
- ($^{++}$) Lorica with irregularly undulate sides; 30–31·5 × 13·5–14·2 μm . . . var. *undulata* var. *nov.*

(e) Lorica broader in the middle and broader than long; neck relatively broad and widened at end; (25·5 –) 28 × (16·7 –) 17 μm . . . 59. *S. rotunda*

(f) Lorica rhomboid
- (i) Hind end elongate with tail not distinct from body; lorica vase-like; 50 × 20 μm . . . . 60. *S. cuneata*
  Lorica smaller; 35 × 15·7 μm . . . . var. *minor* var. *nov.*
- (ii) Hind end not elongate, and with distinct tail
  - ($^+$) Lorica small, 27–33 × 14–20 μm, with short thick tail and relatively long neck . . . . 61. *S. schauinslandii*
  - ($^{++}$) Lorica medium-sized, up to 70 μm long.
    - (*) Tail not disproportionately long; lorica (46 –) 56–70 × (26 –) 30–40 μm . . . . 62. *S. gibberosa*
      - ($^\times$) Lorica swollen with shorter neck and practically no tail; 53 × 39 μm . . . . var. *tumida*
      - ($^{\times\times}$) Neck fairly long and cylindrical, tail also fairly long; lorica (52 –) 53 (– 54·5) × (19·4 –) 20– (21·2) μm . . . . var. *perlonga*
      - ($^{\times\times\times}$) Lorica with equatorial joint but without any swelling in anterior half; tail fairly long and conical; 63–72 × 35–37 μm . . . . var. *kalingensis* var. *nov.*
    - (**) Tail disproportionately long; lorica not markedly ornamented; 44–53 × 22–29 μm; tail 21 μm . . . 63. *S. longicauda*
  - ($^{+++}$) Lorica very long, up to 120–130 μm . . . . 64. *S. ensifera*
    Lorica irregularly verrucose, and smaller; 62 × 35 μm . . . var. *verrucosa* var. *nov.*

(B) Lorica with folds, wrinkles or grooves . . . Subsection 2. *Corrugatae*
Folds transverse resulting in transverse constrictions
Constrictions or wrinkles irregular; Lorica ellipsoid to ovoid; 50–55 × 27–30 μm . . . . 65. *S. tambowika*

II. Lorica in transverse diameter flattened, elliptic in cross section and not angular . . . . Section II. *Complanatae* Conrad
Hind end pointed and without a proper tail; lorica with a few spiral bands and slightly sigmoid; membrane granulate; 50–55 × 24–28 μm; 17 μm thick . . . . . 66. *S. subcurvata*

III. Lorica angular in cross section . . . Section III. *Prismaticae* Conrad 1952
(A) Lorica triangular in cross section . . . Subsection 1. *Triangulares* Huber-Pest. 1955
- (i) Lorica with parallel sides and a tail; 40 × 20 μm . . . . 67. *S. triquetra*

Lorica trapezeoid and with broad front and narrow hind ends; lorica slightly twisted; 35–40 (−42) × 18–22 μm . . . . var. *torta*

(2) Lorica elongate fusiform-rhomboid with anterior and posterior halves triangular; with a medium-sized or long tail

(a) Tail medium-sized, chromatophores small, discoid and without pyrenoids; 55·6–73·8–84 × 26·4–34·4–44 μm; tail alone 10·5–16 μm . . . . 68. *S. reisii*

(b) Tail fairly long; chromatophores large and polyhedral and with diplopyrenoids; lorica 63·5–79·2 × 27–30 μm; tail alone 19·8–27·3 μm . . . . 69. *S. indica* sp. *nov.*

(B) Lorica quadrangular in cross section Subsection 2. *Quadrangulares* Huber-Pest. 1955

Lorica rhomboid and winged in front view and sigmoid in side view; irregularly quadrangular in cross section; with a long (21·2–27·2 μm) straight tail; lorica 68·6 × 31·7–41·5 μm . . . . 70. *S. tortuosa* sp. *nov.*

48. *Strombomonas hexagonalis* Philipose sp. *nov.* (Figura 48)

Lorica plus minusve hexagonalem in forma, habetque in regione media patera recta vel paulo undulata; collo fere absente necnon fistula valde magna margine irregulari; pars posterior in puncto acuto terminat; membranum valde crassum praesertium ad finem posteriorem, fusci coloris pauloque verrucosum; res in cellula non aunt observatae; lorica 37 × 21 μm, cum fistula 7·9–8·8 μm latitudinis.

Habitatio: Aberrans in locatione 6 (mense Iunio) et 92 (mense Aprili).

Typus: Figura 48.

Lorica more or less hexagonal in outline with the sides in median region straight or slightly undulate; neck almost absent and the pore very large and having an irregular margin; hind portion ending in an acute point; membrane very thick especially at the hind end, dirty brown and slightly verrucose; cell contents not observed; lorica 37 × 21 μm with pore 7·9–8·8 μm in breadth.

Habitat: Stray in location 6 (June) and 92 (April).

Type: Figure 48.

Distribution in Indian region: Assam and Orissa (!).

49. *Strombomonas laganaeformis* Huber-Pest. 1955 (figure 49)

Huber-Pestalozzi 1955, p 372, figure 695A.

Lorica fairly large (56·5 × 42 μm with pore 11·3–12·6 μm wide), flask-shaped and ovoid to spherical with thick smooth wall, and hind end broadly rounded and the front ending gradually in a neck which is short and broad, but not distinct; cell contents not observed.

Habitat: Stray in locations 28 and 29 (November–December).

The organism agreed fairly well with Huber-Pestalozzi's species measuring 52–65 × 41·6–43 μm with neck 13 μm broad.

Distribution in Indian region: West Bengal (!).

50. *Strombomonas verrucosa* (Daday) Defl. 1930

Deflandre 1930, p 561, figure 2; Huber-Pestalozzi 1955, p 369, figure 790; = *Trachelomonas hispida* var. *verrucosa* Daday.

Lorica rounded, broadly cylindrical with the base broadly rounded or top-shaped, often also nearly spherical or club-shaped; brownish or yellowish; surface of lorica with irregular granulations or stumpy elevations; lorica narrowed towards the anterior end abruptly into a neck which is obliquely cut or straight or sometimes widened with the margin sometimes toothed; base of lorica rounded and a little arched, slightly bent or pointed or sometimes ending in a short tail up to 12 μm long; chromatophores numerous and discoid; with an eye-spot; flagellum 3-times body length; lorica 20·5–50 × 10–28·8 μm, neck 4·2–7·2 μm broad.

The typical species not observed by the author but reported from Utter Pradesh by Hortobágyi (1969, 36–43 × 20–22 μm, with neck 3–6·5 μm and tail 5–6·5 μm).

var. *conspersa* (Pascher) Deflandre 1930 (figures 50 a–c)

Deflandre 1930, p 567, figures 11–16; Huber-Pestalozzi 1955, pp 370–71, figure 792; = *Trachelomonas conspersa* Pascher *ex* Lemmermann 1913, pp 147–148, figure 273; Deflandre 1926, p 114, figures 612–617.

Lorica broadly cylindrical or sometimes irregularly short-cylindrical with the base broadened and without a tail, and the front end abruptly ending in a neck with the mouth irregularly cut; hind end slightly arched; lorica brownish to yellowish and irregularly granular; lorica 26·4–35·2 × 15–20 μm with the neck 6–6·5 × 5·3–6·5 μm.

Habitat: Stray to rare in locations 29 (April–May), 33 (April, June), 63 (NP 25, 27, March, July), 81 (December); common in location 28 (April, June) and 151 (February, April, July and November); abundant in location 61 (NP 17, May).

The organism agreed well with Pascher's species measuring 25–35 × 10–25 μm with neck 5–7 μm long.

Distribution in Indian region: West Bengal, Orissa and Tamil Nadu (!).

var. *obovata* Philipose var. *nov.* (figurae 50 d–f)

Lorica plus minusve obovoidea, extremitatem posteriorem rotundam et angustiorem, extremitatem vero anteriorem latiorem, necnon in collum latum foramine paululum vel distincte oblique aliquantulum paulatim terminantem; murus loricae aliquantulum undulatus, irregulariter verrucosus flavusque coloris; chromatophora numerosae, parvae sineque pyrenoidis; stigma prominens; flagellum circa unam et quartam partem longitudinis loricae; lorica 33·8–37·5 × 11·3–20·6 μm; collum 6·0–7·2 × 7·2–7·5 μm.

Habitatio: Rara in locatione 151 (mensibus Februarie–Novembri); aberrans in locatione 5 (mense Junio), 83 (mense Decembri) et 134 (mense Novembri).

Lorica more or less obovoid with the hind end rounded and narrower, and the front end broader and ending somewhat gradually in a broad neck with the opening slightly or markedly oblique; wall of lorica somewhat wavy, irregularly verrucose and yellowish brown; chromatophores numerous, small and discoid without pyrenoids; eye-spot prominent; flagellum about 1¼ times the length of lorica; lorica 33·8–37·5 × 11·3–20·6 μm with the neck 6–7·2 × 7·2–7·5 μm.

Habitat: Rare in location 151 (February–November); stray in locations 5 (June), 83 (December) and 134 (November).

Distribution in Indian region: Assam, Orissa, Karnataka and Tamil Nadu (!).

var. *claviformis* (Defl.) Defl. 1930

Deflandre 1930, p 569, figures 20–24; Huber-Pestalozzi 1955, p 371, figure 795; = *Trachelomonas claviformis* Deflandre 1927.

Lorica ovoid and brown, distinctly attenuated towards the front end with a neck cut straight; posterior end broadly rounded and covered with small tubercles; lorica 28 × 16 μm with neck 5–6 μm broad.

The typical variety not observed by the author.

forma *minor* Philipose f. nov. (figure 50g)

Forma a var. typica differens eo quod dimensiones sunt minores necnon tubercula super loricam majora sunt irregulariorque; lorica 21 × 10·6 μm cum fistula 4·4 μm latitudinis; chromatophora circa 9–10 sine pyrenoidis; flagellum breve.

Habitatio: Aberrans in locatione 29 (mense Decembri).

Differs from the typical variety in its smaller dimensions and the tubercles on the lorica being larger and more irregular; lorica 21 × 10·6 μm with the pore 4·4 μm in breadth; chromatophores about 9–10 without pyrenoids; flagellum short.

Habitat: Stray in location 29 (December).

Distribution in Indian region: West Bengal (!).

var. *borysthenienisis* (Roll) Defl. 1930 (figure 50h)

Deflandre 1930, p 568, figure 17; Huber-Pestalozzi 1955, p 371, figure 793; = *Trachelomonas borysthenienisis* Roll 1928.

Lorica ovoid with a short, broad, obliquely cut neck and undulate margin; membrane very thick; light brown and short thick papillae uniformly distributed over the surface; chromatophores numerous, small (about 3 μm), discoid and with double-sheathed pyrenoids; eye-spot oval and fairly large (2·3–3·3 μm); flagellum (observed only in one individual) exceptionally long (about 4 times the length of the lorica); lorica 24–26·3 × 18–19 μm, with neck 2·5 × 7–7·5 μm.

Habitat: Rather common in location 6 (June); very common (April) and rare (July–December) in location 151.

The organism agreed well with Roll's species, though it was slightly smaller, the dimensions given by Roll being 32 × 21·6 μm with pore 8 μm in diameter, eye-spot 3 μm and chromatophores 3·4 μm in diameter. The author's specimens were light brown compared to Roll's specimens which were dark brown and Hortobágyi's (1969) which were colourless.

Distribution in Indian region: Uttar Pradesh (Hortobágyi 1969, 28·6–34 × 20–21 μm, neck 6·5–7 × 2·5–4 μm); Assam and Tamil Nadu (!).

var. *genuina* Defl. 1930 (figure 50i)

Deflandre 1930, p 565, figures 3–5; Huber-Pestalozzi 1955, p 370, figure 790.

Lorica usually small and trapezeoid to ellipsoid with the base often slightly arched and with a very short tail in the form of a pointed or obtuse often oblique papilla; chromatophores numerous, discoid and without pyrenoids; lorica 24·6 × 19·4 μm with neck 3·5 × 5·3 μm and tail 2·6 μm.

Habitat: Stray in location 33 (April) and 81 (December).

Huber-Pestalozzi (1955) states that though Deflandre treated it as a variety, according to normal botanical nomenclature it should not be separated from the typical species since it is not a characteristic form except for its smaller lorica.

Deflandre did not give the dimensions of this variety. There is also a certain degree of overlapping between the figures given by Deflandre for the typical species and this variety. Therefore, there is probably every justification for including it under the typical species.

Distribution in Indian region: West Bengal and Orissa (!).

var. *zmiewika* (Swirenko) Defl. 1930 (figures 50j–l)

Deflandre 1930, p 566, figures 6–10; Huber-Pestalozzi 1955, p 370, figure 791; = *Trachelomonas zmiewika* Swir. 1914; Deflandre 1926, pp 125–26, figures 757, 763 and 764.

Lorica ellipsoid with the front end narrowed into an obliquely cut or straight neck with notched or toothed margin, and with a more or less well developed, pointed, rarely stumpy tail; lorica irregularly granulate to scrobiculate and brown;

chromatophores numerous, discoid and without pyrenoids; flagellum slightly less than the length of the lorica; lorica 37·9–43 × 21–25 μm with neck 5·3–5·8 × 5·3–6·4 μm and tail 6·2–8·1–12·3 μm.

Habitat: Rather common to common in location 151 (April and July) and stray to rare in March, May and August to November; stray in locations 27 (May), 28 (May), 46 (December), 63 (NP 26, September), 82 (December), 117 (December) and in cultures of silt from location 61 (NP 16, September).

Deflandre and Huber-Pestalozzi give the dimensions as 37–50 × 20–27 μm.

Distribution in Indian region: Burma (as *Trachelomonas zmiewika* Swir., Skvortzov 1937, 34–42 × 20–23 μm); West Bengal, Orissa, Andhra Pradesh and Tamil Nadu (!).

51. *Strombomonas urceolata* (Stokes) Deflandre 1930

Deflandre 1930, p 586, figures 70, 71 and 73; Huber-Pestalozzi 1955, p 375, figure 804; = *Trachelomonas urceolata* Stokes 1887, 1888; Playfair 1921, p 134, plate 7, figure 9; *non T. urceolata* Stokes sec. Playf. *et* Defl.- Deflandre 1926, p 126, figures 774–775.

Lorica vase-like, smooth, about twice as long as broad with the sides slightly flattened, and continued somewhat abruptly towards the front end as a short relatively broad neck which is frequently widened and usually cut straight; hind end with a stout pointed and straight tail; chromatophores large, not many and without pyrenoids; lorica 38–57 × 22–28 μm.

The typical species not observed by the author but reported from Maharashtra by Kamat (1975).

var. *elegans* (Playf.) Defl. 1930 (figure 51)

Deflandre 1930, p 588, figure 72; Huber-Pestalozzi 1955, p 375, figure 804A; = *Trachelomonas napiformis* var. *elegans* Playfair 1915; Deflandre 1926, p 124, figure 733; = *T. gickthornii* Skvortzov 1926.

Lorica more or less as in the type but with the sides slightly arched, attenuating abruptly towards the front into a short neck with markedly widened and even margin; hind end converging rapidly into a short, straight, pointed tail; membrane smooth and deep yellow; lorica 58·5 × 29 μm.

Habitat: Stray in locations 19 (April), 58 (November), 63 (NP 26, December), 88 and 157 (December).

The variety agreed fairly well with Playfair's variety measuring 60 × 30 μm.

Distribution in Indian region: Assam, Orissa and Tamil Nadu (!).

52. *Strombomonas vaseformis* Philipose sp. *nov.* (figura 52)

Lorica est elongata in forma necnon hexagonalis habetque finem anteriorem patiorem lateraque paululum depressa in media regione magis subitoque coarctantia in punctum ad finem posteriorem; collum latius est magis quam longum; fistula amplans ad instar vasculi margine undulata vel paululum incisa; membranum paulo asperum necnon flavi coloris; chromatophora numerosae, parvae, discoideasque sineque pyrenoidis; lorica (collo incluso) 27·5 × 10·5 μm; collum 5·3 × 6·8 μm; foramen 8·6 μm.

Habitatio: Aberrans in locatione 151 (mense Aprili).

Typus: Figura 52.

Lorica elongate and subhexagonal with the front end broader, sides slightly depressed in the median region and narrowing rather abruptly to a point at the hind end; neck broader than long, pore broadened like a vase with its margin undulate or slightly incised; membrane slightly rugged and brown; chromatophores numerous, small, discoid and without pyrenoids; lorica (including neck) 27·5 × 10·5 μm; neck 5·3 × 6·8 μm; aperture 8·6 μm broad.

Habitat: Stray in location 151 (April).

Type: Figure 52.

Distribution in Indian region: Tamil Nadu (!).

53. *Strombomonas girardiana* (Playf.) Defl. 1930 (figure 53)

Deflandre 1930, p 573, figures 33–35; Huber-Pestalozzi 1955, p 375, figure 805; = *Trachelomonas urceolata* var. *girardiana* Playfair 1915, p 32, plate 5, figures 7–8; = *T. girardiana* Playfair 1921, p 134; = *T. girardiana* (Playf.) Defl. 1924; 1926, p 126, figures 766, 770.

Lorica subhexagonal in front view and circular in vertical view; sides parallel in the median region, even or slightly depressed; length of neck variable; end of neck straight or oblique, with the margin incised or denticulate; membrane rugged and yellowish; lorica 38·7 × 19·4 μm; tail short or fairly long; chromatophores fairly large and without pyrenoids; eye-spot prominent and curved.

Habitat: In cultures of silt from location 61 (September).

The organism agreed fairly well with the typical species in shape and other features but the lorica was smaller, the typical species measuring 38–57 × 22–26 μm.

Distribution in Indian region: Karnataka (as *Trachelomonas girardiana* (Playf.) Defl., Hegde and Bharati 1986); Orissa (!).

54. *Strombomonas praeliaris* (Palmer) Defl. 1930 (figure 54)

Deflandre 1930, p 578, figures 41–45 and 47–51; Huber-Pestalozzi 1955, p 376, figure 807; = *Trachelomonas praeliaris* Palmer.

Lorica nearly spherical with thin hyaline, more or less colourless membrane which is slightly scabrous and finely punctate; anterior end with a cylindrical neck which is slightly widened towards the tip and obliquely cut with smooth margin; neck also colourless but often indistinctly scabrous; hind end with a fairly long tail which is straight or oblique or sometimes slightly curved and punctate; internal contents of cell not observed; flagellum short; lorica 25–27 μm in diameter; neck 5·3–8·8 × 5·3 μm; tail 12·3–14 μm long.

Habitat: Stray in location 28 (April–May).

Measurements given by Palmer are, lorica 20–28 μm in diameter, neck 5–16 × 5·7 μm and tail 6·5–14 μm long.

Distribution in Indian region: West Bengal (!).

55. *Strombomonas napiformis* (Playf.) Defl. 1930 (figure 55a)

Deflandre 1930, p 590, figure 82; Huber-Pestalozzi 1955, pp 376–77, figure 809; = *Trachelomonas napiformis* Playfair 1915, p 33, plate 5, figure 10; Deflandre 1926, p 124, figure 732.

Lorica oval in the middle with the front end broadened and the hind end narrowed and the flanks rounded; with a short stout pointed tail from the hind end and a cylindrical neck which is nearly as long as broad at the front end; lorica smooth and 32–34 × 17·5–18 μm with neck 7–8 × 6–6·5 μm; tail 6·5 μm.

Habitat: Stray in location 28 (April) and 63 (NP 29, February).

Distribution in Indian region: West Bengal and Orissa (!).

var. *brevicollis* (Playf.) Defl. 1930 (figures 55b, c)

Deflandre 1930, p 591, figures 83–84; Huber-Pestalozzi 1955, p 377, figure 810; = *Trachelomonas napiformis* Playf. var. *brevicollis* Playf. 1921, p 135, plate 7, figures 12–14.

Lorica more markedly ovoid than in the type with rather short neck which widens like a vase, but with a longer tail; chromatophores (reported by Playfair as fairly large) small, numerous and discoid; flagellum shorter than the lorica; lorica 45–46·7 × 24·6–27 μm, neck 4–4·4 × 6–6·2 μm and tail 10·6 μm.

Habitat: Stray in locations 19 (April) and 157 (December), rare in locations 27 (May) and 88 (December) and rather common in location 28 (April); also stray in cultures of silt from location 151 (April–June and October–December).

The variety agreed well with the Australian organism measuring 48–53 × 24–25 μm (middle of lorica 36–38 μm long), neck 5–6 × 6–11 μm and tail 10–14 μm, but the eye-spot shown as discoid by Playfair was much longer and somewhat curved and triangular in the specimen observed from location 151.

Distribution in Indian region: Maharashtra (Kamat 1975); Assam, West Bengal, Orissa and Tamil Nadu (!).

56. *Strombomonas ovalis* (Playf.) Defl. 1930 (figure 56a)

Deflandre 1930, p 577, figures 42–43; Huber-Pestalozzi 1955, pp 377–78, figure 811; —*Trachelomonas urceolata* var. *ovalis* Playfair 1915, plate 5, figures 5–6; Deflandre 1926, p 127, figures 771 and 773.

Lorica regularly ellipsoid with a short obliquely cut neck which is slightly widened towards the tip and with smooth margin; hind end rapidly narrowed to a short pointed tail; membrane smooth; lorica 30–35 × 20–21·2 μm; neck 4·4–6·2 × 6–6·5 μm; tail 5·3–6 μm long.

Habitat: Stray in locations 19 (April), 63 (NP 27, March), 103 (December), 125 (February) and 150 (February).

It agreed well with Playfair's species measuring 34–37 × 22 μm with neck 4–6 × 6 μm and tail 6 μm.

Distribution in Indian region: Maharashtra (Kamat 1975), Assam, Orissa, Andhra Pradesh and Kerala (!).

forma *minor* Philipose f. *nov.* (figura 56b)

Lorica est minoris magnitudinis quam species typica in dimensione tantum 23 μm (collo caudaque inclusis) × 14 μm, cum collo 3·4 × 4·2 μm necnon cauda 5·2 μm; collum paulo latius est ad apicem labrumque habet; chromatophora nermerosae et discoideae.

Habitatio: Aberrans in locatione 151 (mense Aprili).

Smaller than the typical species, measuring only 23 μm (including neck and tail) × 14 μm with the neck 3·4 × 4·2 μm and tail 5·2 μm; neck wider towards the tip and with a rim.

Habitat: Stray in location 151 (April).

Distribution in Indian region: Tamil Nadu (!).

57. *Strombomonas madraspatensis* sp. *nov.* (figurae 57a, b)

Forma loricae est ablate ellipsoidea ad ovoideam varians habetque finem anteriorem prolongatam in breve crassumque collum cum fistula recta necnon margine regulariter dentata; finis posterior habet caudam brevem, truncam necnon asperum; membranum asperum est fulvique coloris; chromatophora numerosae, discoideae sunt sine pyrenoidis; grans paramyli parva est necnon ad instar virgulae in forma; flagellum breve; lorica 41·4–43 × 23·7–24·3 μm, collum vero 5·7–6 μm necnon cauda 9·3 μm.

Habitatio: Rara in locatione 151 (mense Aprili).

Typus: Figurae 57a, b.

Lorica broadly ellipsoid to ovoid with the anterior end produced into a short stout neck with the pore straight and with a regularly dentate margin; posterior end with a short, stumpy, rugged tail; membrane rugged and yellowish brown; chromatophores numerous, discoid and without pyrenoids; paramylum small and rod-shaped; flagellum short; lorica 41·4–43 × 23·7–24·3 μm, neck 5·7 × 6 μm and tail 9·3 μm.

Habitat: Rare in location 151 (April).

Type: Figure 57a, b.

Though the organism resembled *S. verrucosa* var. *zmiewika* in size, the lorica was ovoid and not trapezeoid, the free margin of the neck was regularly dentate and the tail stout, stumpy, and not pointed. It also showed some resemblance to *S. deflandrei* (see Huber-Pestalozzi 1955, p 378, figure 12) in shape and some other features, but the neck was longer, the free margin of the neck was not irregular, the membrane of the lorica not wrinkled and the tail longer and not pointed, the dimensions of *S. deflandrei* being 40·5 × 24·3 μm with neck 3·7 × 6·8 μm and tail 2·7 μm. So it is treated as a new species and placed near *S. deflandrei.*

Distribution in Indian region: Tamil Nadu (!).

58. *Strombomonas fluviatilis* (Lemm.) Defl. 1930 (figure 58a)

Deflandre 1930, p 580, figures 52–53; Huber-Pestalozzi 1955, pp 378–79, figure 815; Hortobágyi 1957, p 8, figure 105, 107–108; = *Trachelomonas fluviatilis* Lemm. 1909; 1913; p 154, figure 278; Deflandre 1926, p 123, figures 720 and 727.

Lorica ellipsoid to broadly spindle-shaped with a narrow or fairly broad neck which is cut straight or obliquely; hind end abruptly narrowed into a short straight or slightly bent tail; membrane light brown and slightly rugged due to small or large verrucae; lorica 30·8 × 16 μm with neck alone 5·3 × 4·4–5·3 μm and tail 2·6–4·4 μm.

Habitat: Stray in location 14 (December).

The organism agreed well with Lemmermann's species measuring 28–38 × 12–17 μm. However, Hortobágyi (1957, 1969, p 32, figure 76) give the dimensions as 25–31·5 × 12·4–19 μm.

Distribution in Indian region: Burma (Skvortzov 1937, p 83, 23·8 × 10·2 μm, neck 2·5 μm broad, as *Trachelomonas fluviatilis* Lemm.); Uttar Pradesh (Hortobágyi 1969, 29–31·5 × 14·2–19 μm); Kerala (Suxena *et al* 1973); Maharashtra (Kamat 1975) and Assam (!).

var. *undulata* Philipose var. nov. (figurae 58 b, c)

Varietas a species typica differens eo quod latera loricae sunt irregulariter undulata;

lorica 26·4–31·5 × 12·3–14·4 μm, cum collo 5·3–6·0 × 4·4–6 μm necnon cauda 2·6–3·0 μm.

Habitatio: Aberrans in locatione 23 (mense Iunio) necnon testinis piscium ex locatione 29 (mense Aprili).

Differs from the typical species in the sides of the lorica being irregularly undulate; lorica 26·4–31·5 × 12·3–14·4 μm with neck 5·3–6 × 4·4–6 μm and tail 2·6–3 μm.

Habitat: Stray in location 23 (June) and in the gut contents of fish from location 29 (April).

Distribution in Indian region: West Bengal (!).

var. *levis* (Lemm.) Defl. 1930 (figure 58d)

Deflandre 1930, p 581, figures 55–56; Huber-Pestalozzi 1955, p 379, figure 817; = *Trachelomonas fluviatilis* var. *levis* (Lemm.) Skvortzov (1925) 1926; = *Trachelomonas affinis* Lemm. 1905; Deflandre 1926, p 127, figure 784.

Differs from the typical species in its larger dimensions; membrane smooth; lorica 63·4 μm long.

Habitat: Stray in location 58 (November).

The organism agreed with Lemmermann's variety which is 60–68·5 μm in length.

Distribution in Indian region: Orissa (!).

59. *Strombomonas rotunda* (Playf.) Defl. 1930 (figure 59)

Deflandre 1930, p 593, figure 58; Huber-Pestalozzi 1955, p 380, figure 821; = *Trachelomonas gibberosa* var. *rotunda* Playfair 1915, plate 5, figure 19; Deflandre 1926, p 128, figure 736.

Lorica transversely ellipsoid with the body broader than long; with a relatively large straight neck having parallel sides and widened like a vase at the end which is smooth; tail fairly long, stout and obtuse; chromatophores small, numerous discoid and without pyrenoids; flagellum shorter than the lorica; lorica 25·5 × 16·7 μm; neck 5·3–7 × 5·3 μm; tail 9·7 μm long.

Habitat: Stray in locations 5d (April), 19 (April), 27 (May), 61 (NP 15, May), 108 (December), 150 (February) and 151 (February).

The organism agreed fairly well with Playfair's species which measures 28 × 17 μm, with neck 8 × 6 μm and tail 10 μm.

Distribution in Indian region: Assam, West Bengal, Orissa, Andhra Pradesh, Kerala and Tamil Nadu (!).

60. *Strombomonas cuneata* (Playf.) Defl. 1930

Deflandre 1930, p 594, figure 89; Huber-Pestalozzi 1955, p 381, figure 826; = *Trachelomonas cuneata* Playf. 1921, p 135, plate 7, figure 15.

Lorica trapezeoid with the sides nearly straight or arched and converging towards a cuneiform posterior; anterior part subtriangular and narrowing to a neck with a vase-like end having a smooth margin; membrane hyaline and scabrous; lorica 50 × 20 μm, neck 6 μm broad and tail 14 μm long.
Known only from Australia.

var. *minor* Philipose var. *nov.* (figura 60)

Varietas a species typica differens ob dimensiones minores, videlicet, lorica 42 μm (collo caudaque inclusis) × 15·7 μm, cum collo sole 6·4 μm longitudinis necnon 7 μm ad fistulum, ore quoque irregulariter incisa; lorica varians in forma ab leni ad paulo scabram; chromatophora 6–8 discoideae sineque pyrenoidis; grana paramyli sunt parva necnon virgulata.

Habitatio: Aberrans in locatione 151 (mensibus Aprili–Iunio, Augusto–Octobri).

Differs from the typical species in its smaller dimensions, viz. lorica 42 μm (including neck and tail) × 15·7 μm, with neck alone 6·4 μm long and 7 μm broad at the pore; mouth also irregularly incised; lorica smooth to slightly scabrous; chromatophores 6–8, discoid and without pyrenoids; paramylum small and rod-like.

Habitat: Stray in location 151 (April–June, August–October).

Distribution in Indian region: Tamil Nadu (!).

61. *Strombomonas schauinslandii* (Lemm.) Defl. 1930 (figures 61a,b)

Deflandre 1930, p 594, figures 90–96; Huber-Pestalozzi 1955, pp 381–82, figure 827; = *Trachelomonas schauinslandii* Lemm. 1904; 1913, p 154, figure 305; Deflandre 1926, p 129, figure 779.

Lorica cuneiform with the sides rounded, gradually tapering to the hind end as a short tail, and converging rapidly to the front as a cylindrical straight fairly long neck which is cut straight and with smooth margin; membrane scabrous; lorica 26–32 × 13·5–17·5 μm.

Habitat: Stray in locations 28 (April, July–August), 33 (April), 61 (NP 20, May) and 109 (December).
Lemmermann's species measured 27–33 × 14–20 μm.

Distribution in Indian region: West Bengal, Orissa and Andhra Pradesh (!).

62. *Strombomonas gibberosa* (Playf.) Defl. 1930 (figures 62a–c)

Deflandre 1930, p 595, figures 97–101 and 103; Huber-Pestalozzi 1955, p 382, figure 828; = *Trachelomonas gibberosa* Playfair 1915, plate 5, figure 16; Deflandre 1926, p 128, figure 759.

Lorica broadly rhomboid with the median region somewhat angular, sides nearly straight or slightly concave or convex; converging rapidly to the front as a neck and to the hind end as a tail; neck usually obliquely cut but sometimes widened like a vase towards the pore; tail hollow, straight or sometimes slightly oblique and pointed; generally with a thin transverse membrane at its base; membrane smooth or rugged; chromatophores small, numerous, discoid and without pyrenoids in material from locations 23 and 27; lorica 46–53–65 × 26·4–29·3–36·7 μm; neck 2·6–3 × 7 μm; tail alone 8·8–12·3 μm.

Habitat: Rare in locations 23 (June), 27 (May); stray in locations 4 (May), 61 (NP 11, May), 63 (NP 25, March), 103 (December) and 109 (December).

The organism from various locations agreed fairly well with the typical species, but the transverse membrane at the base of the tail was not always observed.

Distribution in Indian region: Assam, West Bengal, Orissa and Andhra Pradesh (!).

var. *tumida* (Playf.) Defl. 1930 (figure 62d)

Deflandre 1930, p 598, figure 107; Huber-Pestalozzi 1955, p 383, figure 833; = *Trachelomonas gibberosa* Playf. var. *tumida* Playfair, 1921, p 135, plate 7, figure 17.

Differs from the typical species in the neck being short and the tail almost absent; instead the sides of the lorica converge towards the posterior and join sharply to form a small projection; lorica 52 × 38·7 μm; neck 7–8 μm long, with the caudal point up to 5 μm.

Habitat: Stray in location 103 (December).

The organism agreed fairly well with Playfair's variety measuring 53 × 39 μm with the neck 7 μm and tail 3 μm, but the tail was slightly longer and there was a transverse partition at the base of the tail, as in the typical species.

Distribution in the Indian region: Andhra Pradesh (!).

var. *perlonga* Defl. 1930 (figure 62e)

Deflandre 1930, p 597, figure 104; Huber-Pestalozzi 1955, p 382, figure 830; = *Trachelomonas ensifera* Drezepolski 1925, Tafel II, figure 102.

Shape of the lorica as in the typical species, but with a very long cylindrical neck and a long stout pointed tail; lorica 52–54·5 × 19·4–21·2 μm.

Deflandre's variety measured 52 × 20 μm.

Distribution in Indian region: Andhra Pradesh (!).

var. ***kalingensis*** Philipose var. ***nov.*** (figures 62f, g)

Varietas a species typica differens eo quod cauda non est aperte separata nec acute cuneiformis necnon ob praesertiam nodi vel cardinis in regione media; membranum transversum absente ad radicem caudae; lorica paulo flavi coloris glabraque; chromatophora numerosae, parvae, discoideae sineque pyrenoidis; lorica 68·4–70·4 × 35–37 μm, collo solo 7·9–8·3 μm, caudaque circa 19·4–23·5 μm.

Habitatio: Rara in locatione 92 (mense Aprili).

Differs from the typical species in the tail being not clear cut and sharply conical, and in the presence of a joint or hinge in the equatorial region; a transverse membrane absent at the base of the tail; lorica light brown and smooth; chromatophores numerous, small, discoid and without pyrenoids; lorica 68·4–70·4 × 35–37 μm with neck alone 7·9 × 8·3 μm and tail about 19·4–23·5 μm.

Habitat: Rare in location 92 (April).

Distribution in Indian region: Orissa (!).

63. ***Strombomonas longicauda*** (Swir.) Defl. 1930 (figure 63)

Deflandre 1930, p 599, figures 108–109; Huber-Pestalozzi 1955, pp 383–84, figure 834; = *Trachelomonas longicauda* Swirenko 1914; Deflandre 1926, p 129, figures 751–752.

Lorica rhomboidal with rounded angles; sides converging rapidly towards the neck as well as towards the tail; neck with the end widened and obliquely cut with the margin indistinctly denticulate; tail long, conical and straight or oblique; membrane brownish, slightly wrinkled and sometimes granular; chromatophores not observed; lorica (total) 52 × 27–28 μm, neck 10·6–11·4 × 6·5–7 μm and tail 19·4–21·2 μm.

Habitat: Stray in locations 61 (NP 11, May) and 102 (December).

Deflandre and Huber-Pestalozzi give dimensions as 44–53 × 20–29 μm with neck 12 × 7 μm and tail 21 μm.

Distribution in Indian region: Orissa and Andhra Pradesh (!).

64. ***Strombomonas ensifera*** (Daday) Defl. 1930 (figure 64a)

Deflandre 1930, p 601, figures 111–113 and 115; Huber-Pestalozzi 1955, p 384, figure 835; = *Trachelomonas ensifera* Daday 1905; Lemmermann 1913, p 154, figure 304; = *T. ensifera* Daday emend. Defl. 1926, p 127, figures 743–744 only.

Lorica very large, rhomboid in the median region and angled or sometimes rounded, narrowing towards the front end into a fairly long obliquely cut neck and towards the hind end into a long, stout, hollow and pointed tail separated from the body by a transverse membrane; membrane smooth, clear brown, relatively thin and slightly deformed; chromatophores not observed; lorica 121–125 × 45–48 μm, with neck 30 × 8 μm and tail 43–48 μm.

Habitat: Stray in locations 6 (June), 61 (NP 11, May) and 75 (December).

It agreed with the typical species measuring 120–134 × 38–55 μm with neck 7–10 μm broad and tail 42–70 μm long.

Distribution in Indian region: Assam and Orissa (!).

var. *verrucosa* Philipose var. *nov.* (figure 64b)

Varietas a species typica differens eo quod lorica est formae irregularis ab rugata ad verrucosam necnon habet dimensiones minores, vidilicet, 61·6 × 35 μm, collum vero 15 × 7 μm, fistulam 8·8 μm diametri necnon caudam 26·4 μm longitudinis; lorica est paulo fulvi coloris; chromatophora numerosae, discoideae sineque pyrenoidis; stigma prominens; flagellum brevius quam lorica ipsa.

Habitatio: Aberrans in locatione 61 (NP 13, mense Iulio).

Differs from the typical species in the lorica being irregularly wrinkled to verrucose and the smaller dimensions, viz. 61·6 × 35 μm with the neck 15 × 7 μm, pore 8·8 μm in diameter and the tail 26·4 μm long; lorica light brown; chromatophores numerous, discoid and without pyrenoids; stigma prominent; flagellum shorter than the lorica.

Habitat: Stray in location 61 (NP 13, July).

Hortobágyi (1957, p 8, figure 142) has recorded a fairly similar form from Hungary as *Trachelomonas ensifera* Daday. Apart from the organism being distinctly granular to verrucose the dimensions are also smaller, viz. 53–56 × 25–33 μm with the pore 6 μm whereas in the typical species of *Strombomonas ensifera* (= *Trachelomonas ensifera*) the dimensions are much larger (see above) and the lorica is smooth and slightly deformed. So, Hortobágyi's organism appears to be the same as the one from Cuttack.

Distribution in Indian region: Orissa (!).

65. *Strombomonas tambowika* (Swir.) Defl. 1930 (figure 65)

Deflandre 1930, p 576, figures 38–41; Huber-Pestalozzi 1955, p 386, figure 842; = *Trachelomonas tambowika* Swir. 1914; Deflandre 1926, p 123, figures 734–735.

Lorica ellipsoid or ovoid with the front end prolonged into a straight neck with denticulate margin; hind end with a long, straight or slightly bent tail; membrane yellowish brown, smooth but with irregular constrictions or wrinkles; lorica 48 × 30 μm.

Habitat: Very rare in location 28 (May).

Swirenko's species measured 50–55 × 27–30 μm.

Distribution in Indian region: West Bengal (!).

66. *Strombomonas subcurvata* (Proschk-Lawr.) Defl. 1930 (figure 66)

Deflandre 1930, p 605, figures 125–126; Huber-Pestalozzi 1955, pp 387–88, figure 845; = *Trachelomonas subcurvata* Proschkina-Lawrenko 1924.

Lorica suboval, slightly compressed, spirally recurved to slightly sigmoid with the hind end acuminate; neck widened towards the pore and with denticulate margin; membrane hyaline with irregularly arranged large granules; cell contents not observed; lorica 48·6 × 27 μm with neck 6·2–7 × 6 μm.

Habitat: Stray in location 63 (NP 25, January).

The organism conformed fairly well to Proschkina-Lawrenko's species measuring 50–55 × 24–28 μm with neck 4·8–8·4 × 6–7·2 μm.

Distribution in Indian region: Orissa (!).

67. *Strombomonas triquetra* (Playf.) Defl. 1930

Deflandre 1930, p 602, figures 116–117; Huber-Pestalozzi 1955, pp 388–89, figure 846; = *Trachelomonas triquetra* Playfair 1915, plate 5, figure 15; Deflandre 1926, p 129, figure 777.

Lorica triangular in cross section with the upper portion of the body rectangular in side view with the sides straight and parallel; lower part of the body rapidly converging into a tail; neck straight and short with a truncate and not widened end; membrane finely scrobicular; lorica 40 × 20 μm.

Known only from Australia.

var. *torta* Rino (figures 67a–d)

Rino 1972, p 169, plate 9, figures 7–10.

Differs from the typical species in the lorica being contorted and trapezeoid in side view with the anterior end slightly broader, sides nearly straight or more or less constricted and attenuated towards the posterior end into a short pointed tail or rarely with the hind end obtuse to rounded and without a tail; anterior end abruptly attenuated into a short broad obliquely cut neck with a smooth margin; chromatophores numerous and discoid; paramylum either few and rod-like or sometimes absent; lorica 36–42 × 18·9–22 μm, with neck 4·4–5·3 × 6·9–7·5 μm; tail when present about 1·8–3·5 μm: eye-spot not observed; flagellum less than body length.

Habitat: Rather common in location 27 (May) and stray in locations 2 (June), 151 (May) and 159 (December).

The organism agreed fairly well with Rino's species measuring 35–40 × 18–22 μm, but in the Indian material the tail was not always present. Rino's specimens had also a flagellum of body length.

Distribution in Indian region: Assam, West Bengal and Tamil Nadu (!).

68. *Strombomonas reisii* Rino 1972 (figures 68a–d)

Rino 1972, p 168, plate 9, figures 5–6.

Lorica elongate, about twice as long as broad, fusiform-rhomboid with the median region angular; posterior half of lorica triangular with somewhat convex sides and narrowing into a medium-sized tail at the hind end; anterior half of lorica also triangular but with slightly concave, rarely slightly convex sides and converging towards the apex to terminate in a short obliquely cut neck with the pore having smooth or slightly irregular margin; in vertical section circular in median region, and triangular seen from anterior and posterior ends; membrane sparsely or densely granular; chromatophores numerous, small, discoid and without pyrenoids; paramylum small and rod-like; eye-spot triangular; flagellum less than body length; lorica (total) 55·6–73·8 × 26·4–34·4 μm, with neck 4·4 × 7–8·6 μm and tail 10·6–14·6–16 μm.

Habitat: Rather common in location 27 (May) and stray to rare in location 151 (May, November).

The organism agreed with Rino's species measuring 84 × 44 μm, but was slightly smaller. The granulation of the lorica was also sometimes more prominent and crowded. Rino did not observe a flagellum and eye-spot.

Distribution in Indian region : West Bengal and Tamil Nadu (!).

69. *Strombomonas indica* Philipose sp. *nov.* (figurae 69a–d)

Lorica elongate, fusiform-rhomboidea necnon triquetra; anterior posteriorque dimidiae partes loricae aunt formae triangulae cum lateribus convexis vel paululum concavis; finis anterior habet brevem, latum, oblique sectile collum cum fistula triangula habens marginem glabrum vel paulo irregularem; finis posterior prolongata est in caudam satis longam, de more rectam, aliquando paululum curvatum acutamque; a fronte posteriorique extremitate visa, lorica est triangula; membranum irregulariter granulatum videtur; chromatophora numerosae, largiores polyhedralesque aunt necnon cum pyrenoidis projicientibus dupliciterque vaginatis; stigma elongatum ad instar disci; flagellum brevius quam lorica; lorica (tota) 63·5–70·5 × 27–28 μm, colle 3·5–4·4 × 5·7–7·2 μm necnon cauda 19·8–24·6 μm.

Habitatio: Rara in locatione 61 (NP 9 et 16, mense Maio).

Typus: Figurae 69a–d.

Lorica elongate, fusiform-rhomboid and triquetrate; anterior and posterior halves of lorica triangular in outline with the sides convex or slightly concave; anterior end with a short broad obliquely cut neck with triangular pore having smooth or slightly irregular margin; posterior end prolonged into a fairly long, usually straight, sometimes very slightly bent, pointed tail; viewed from front and hind ends lorica triangular; membrane irregularly granulate; chromatophores numerous, fairly large and polyhedral with double-sheathed projecting pyrenoids; eye-spot elongate and disc-like; flagellum shorter than the lorica; lorica (total) 63·5–70·5 × 27–28 μm, neck 3·5–4·4 × 5·7–7·2 μm and tail 19·8–24·6 μm.

Habitat: Rare in location 61 (NP 9 and 16, May).

Type: Figures 69a–d.

Distribution in Indian region: Orissa (!).

70. *Strombomonas tortuosa* Philipose sp. *nov.* (figurae 70a–c)

Lorica rhomboidea, aligerque a fronte, necnon sigmoidea ab latere visa, irregulariter quadrangula in sectione optica dicte; habet breve, latum oblique sectile collum ad finem anteriorem necnon longiorem de more rectam caudam ad finem vero posteriorem; membranum irregulariter punctata ad granulata; lorica 65·0–73·2 × 29·9–33·2 μm cum collo 3·5–6·2 × 7–7·9 μm; fistula 7–8·8 μm diametri necnon cauda 21·2–27·3 μm longitudinis; res internae non sunt observatae.

Habitatio: Aberrans in locationibus 61 (NP 16, mense Decembri) et 92 (mense Aprili).

Typus: Figurae 70a–c.

Lorica rhomboid and winged in front view and sigmoid in side view, irregularly quadrangular in optical cross section; with a short wide obliquely cut neck in front end and with a fairly long usually straight tail at the hind end; membrane irregularly punctate to granulate; lorica 65–73·2 × 29·9–33·2 μm with neck 3·5–6·2 × 7–7·9 μm, pore 7–8·8 μm in diameter and tail 21·2–27·3 μm long; internal contents not observed.

Habitat: Stray in locations 61 (NP 16, December) and 92 (April).

Type: Figures 70a–c.

Though the organism showed some resemblance to *S. reisii* and *S. indica* in size and general shape it had a sigmoid lorica which was irregularly quadrangular and not triquetrate in cross section and the tail was longer.

Distribution in Indian region: Orissa (!).

Other taxa of *Strombomonas* reported from the Indian region are as follows:

1) *S. australica* (Playf.) Defl. 1930 (= *Trachelomonas caudata* var. *australica* Playf. 915); = *T. longissima* Defl. 1926 (Burma, Skuja 1949, p 165).
2) *S. gibberosa* Playf. 1915 var. *indica* Skvortzov 1937 (Rangoon, Skvortzov 1937, 84).
3) *S. maxima* (Skv.) Defl. 1930; Vidarbh (Kamat 1975, p 468).
4) *S. urceolata* (Stokes) Defl. 1930; Vidarbh (Kamat 1975, p 468).
5) *S. urceolata* (Stokes) Defl. var. *indica* Skvortzov 1937; Burma (Skvortzov 1937, 84).
6) *S. urceolata* (Stokes) Defl. var. *kolhapurensis* Kamat 1963; Kolhapur (Kamat 963, p 267).
7) *S. verrucosa* (Daday) Defl. 1930; United Provinces (Hortobágyi 1969, p 32).

Skvortzov's (1937, p 82) *Trachelomonas rangoonensis* and *T. rangoonensis* var. *urvata* could most probably be only a *Strombomonas verrucosa* var. *borystheniensis* Roll) Defl. 1930.

cknowledgements

he author is deeply grateful to Father H Macheda, Theodore's Sanatorium, Vellington Barracks, Nilgiris, for the latin translations of descriptions of new taxa nd Dr M R Suxena, Emeritus Professor, Osmania University, Hyderabad, for help obtaining some literature. Thanks are due to S Radhakrishnan, Central Inland isheries, Assam Unit, Joysagar, who kindly made pencil sketches of figures 7, 13a,b, 0–22a,c, 23, 25, 29, 30b, 32d, 35 and 58a, from specimens collected by him in and round Joysagar and Nazira, Assam.

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Figures 1–70. **1. a and b.** *Trachelomonas volvocina* Ehr. **c.** *T. volvocina* var. *planctonica* Playf. **2.** *T. volvocinopsis* Swir. **3.** *T. varians* Defl. **4.** *T. sparsesetulosa* Huber-Pest. **5.** *T. verrucosa* var. *macrotuberculata* Grandori. **6.** *T. costatus* sp. *nov.* **7.** *T. assamensis* Phil. et. Radakr. sp. *nov.* **8.** *T. heduma* Conrad. **9.** *T. curta* Da Cunha emend. Defl. **10.** *T. lismorensis* var. *biseriata* Playf. **11.** *T. tuberculata* Middel. var. *irregularis* var. *nov.* **12. a.** *T. oblonga* Lemm. **b.** *T. oblonga* var. *australica* Playf. **13. a.** *T. mucosa* Swir. var. *sphaerica* Phil. *et* Radhakr. var. *nov.* **b.** *T. mucosa* Swir. var. *subglobosa* Phil. *et* Radhakr. var. *nov.* **14. a.** *T. intermedia* Dang. **b.** *T. intermedia* Dang. f. *major* forma *nov.* **15.** *T. neotropica* Balech. **16.** *T. ovata* Roll. **17. a.** *T. cylindrica* Playf. **b.** *T. cylindrica* var. *decollata* Playf. **18. a and b.** *T. hispida* (Perty) Stein emend. Defl. **c and d.** *T. hispida* var. *crenulatocollis* (Muskell) Lemm. **e.** *T. hispida* var. *crenulatocollis* f. *glabra* forma nov. **f.** *Trachelomonas hispida* var. *crenulatocollis* forma *recta* Defl. **g.** *T. hispida* var. *coronata* Lemm. **19.** *T. allia* Drez. **20.** *T. joysagarensis* Phil. et Radakr. sp. *nov.* **21.** *T. kelloggii* Skv. emend. Defl. **22. a and b.** *T. bacillifera* var. *minima* Playf. **c.** *T. bacillifera* var. *sparsespina* Defl. **23.** *T. robusta* Swir. emend. Defl. var. *major* Phil. et Radhakr. var. *nov.* **24. a.** *T. superba* Swir. emend. Defl. **b.** *T. superba* var. *coronata* var. *nov.* **25.** *T. horrida* Palmer var. *moenacanthum* Skv. **26. a.** *T. armata* (Ehr.) stein; **b.** *T. armata* var. *steinii* Lemm. emend. Defl. and **c.** forma *punctata* (Swirenko) Defl.; **d and e.** *T. armata* var. *duplex* forma *jorhatensis* f. nov.; **f.** *T. armata* var. *malabarica* var. nov.; **g.** *T. armata* var. *longispina* Playf. emend. Defl. forma *punctata* f. nov.; **h, i and j.** *T. armata* var. *longa* Defl. **27. a.** *T. dangeardiana* (Defl.) Huber-Pest. var. *glabra* (Playf.) Defl. **b.** *T. dangeardiana* var. *cylindrica* var. *nov.* **28.** *T. eurystoma* Stein Sec. Playf. var. *nuda* Szabadoz. **29.** *T. zorensis* Defl. **30. a.** *T. abrupta* Swir. emend. Defl. var. *minor* Defl. **b.** *T. abrupta* var. *arcuata* (Playf.) Defl. **31. a.** *T. granulosa* Playf. **b.** *T. granulosa* var. *crenulatocollis* (Szab.) Huber-Pest. forma *obovata* f. *nov.* **32. a.** *T. scabra* Playf. **b.** *T. scabra* var. *longicollis* Playf. **c.** *T. scabra* var. *coberensis* Defl. **d.** *T. scabra* var. *labiata* (Teiling) Huber-Pest. **33.** *T. szabadosiana* Huber-Pest. **f.** *minor* forma *nov.* **34.** *T. aspera* Da Cunha. **35.** *T. planctonica* Swirenko var. *minor* Phil. et Radhakr. forma *nov.* **36.** *T. volzii* Lemm. var. *cylindracea* Playf. **37.** *T. dubia* Swir. emend. Defl. var. *lata* Defl. **38.** *T. hexangulata* (Swir.) Playf. **39.** *T. bulla* Stein emend. Defl. **40. a.** *T. playfairii* Defl. **b.** *T. playfairii* var. *hyalina* (Defl.) Hortob. **41. a and b.** *T. similis* Stokes. **42.** *T. atrata* (Skv.) Defl. var. *pustulosa* Conrad. **43.** *T. gregusii* Hortob. **44.** *T. pseudocaudata* var. *elongata* var. *nov.* **45.** *T. bernardinensis* Vischer. **46. a.** *T. helvetica* Lemm. emend. Defl. var. *elongata* Bourr. **b.** *T. helvetica* var. *cucurbita* (Skv.) Huber-pest. **47. a and b.** *T. nadsonii* Skv. var. *indica* Skv. f. *kausalyagangensis* forma *nov.* **48.** *Strombomonas hexagonalis* sp. *nov.* **49.** *S. laganaeformis* Huber-Pest. **50. a–c.** *S. verrucosa* (Daday) Defl. var. *conspersa* (Pasch.) Defl. **d–f.** *S. verrucosa* var. *obovata* var. *nov.* **g.** *S. verrucosa* var. *claviformis* (Defl.) Defl. f. *minor* forma *nov.* **h.** *S. borystheniensis* (Roll) Defl. **i.** *S. verrucosa* var. *genuina* Defl. **j–l.** *S. verrucosa* var. *zmiewika* (Swir.) Defl. **51.** *S. urceolata* (Stokes) Defl. var. *elegans* (Playf.) Defl. **52.** *S. vaseformis* sp. *nov.* **53.** *S. girardiana* (Playf.) Defl. **54.** *S. praeliaris* (Palmer) Defl. **55. a.** *S. napiformis* (Playf.) Defl. **b and c.** *S. napiformis* var. *brevicollis* (Playf.) Defl. **56. a.** *S. ovalis* (Playf.) Defl. **b.** *S. ovalis* f. *minor* f. *nov.* **57.** *S. madraspatensis* sp. *nov.* **58. a.** *S. fluviatilis* (Lemm.) Defl. **b and c.** *Strombomonas fluviatilis* var. *undulata* var. *nov.* **d.** *S. fluviatilis* var. *levis* (Lemm.) Defl. **59.** *S. rotunda* (Playf.) Defl. **60.** *S. cuneata* (Playf.) Defl. var. *minor* var. *nov.* **61. a and b.** *S. schauinslandii* (Lemm.) Defl. **62. a–c.** *S. gibberosa* (Playf.) Defl. **d.** *S. gibberosa* var. *tumida* (Playf.) Defl. **e.** *S. gibberosa* var. *perlonga* Defl. **f and g.** *S. gibberosa* var. *kalingensis* var. *nov.* **63.** *S. longicauda* (Swir.) Defl. **64. a.**

S. ensifera (Daday) Defl. **b.** *S. ensifera* var. *verrucosa* var. *nov.* **65.** *S. tambowika* (Swir.) Defl. **66.** *S. subcurvata* (Prosch-Lawr.) Defl. **67. a–d.** *S. triquetra* (Playf.) Defl. var. *torta* Rino. **68. a.** *S. reisii* Rino. **b and c.** *Strombomonas reisii* Rino. **d.** Optical cross section of same. **69. a.** *S. indica* sp. *nov.* **b and c.** *S. indica* sp. *nov.* **d.** optical cross section of the same from tail end. **70. a–c.** Three views of the *S. tortuosa* sp. *nov.*
Same magnification—(18g, 24b, 26a and f–i); (19a and b, 25, 26b); (20, 22a–c); (26j, 27b, 31b, 32a, 36a and b, 44, 47a and b); (28, 37, 46a and b); (26j, 30b, 31a, 32d); (32b, 32c); (33, 34, 38, 42, 43); (48, 50g, i, k and l; 53, 55c); (50a–c); (50d–f, 50h and j, 57a and b); (52, 56b); (58b and c, 59, 61a and b, 62b and c, 67a–d, 68b); (58d, 62f and g); (62e, 63); (62d, 64a); (69b and c, 70a–c).

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 5, October 1988, pp. 395–398.
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Cytological studies in *Launaea pinnatifida* Cass

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MS received 20 July 1987; revised 6 September 1988

Abstract. *Launaea pinnatifida* Cass. a member of the compositae, is a runner growing on sandy sea coasts of Indian subcontinent. The plant bears yellow flowered heads and produces milky latex. The cytological study with respect to its geographical distribution has been carried out. All the collections of *Launaea pinnatifida* showed $2n = 18$ chromosomes. Nine bivalents are counted at diakinesis. Meiosis is normal. One or two nucleolar bivalents are noticed. Position of centromeres in the chromosomes showed variation, this may be due to structural rearrangement taking place in the chromosomes of the taxon which has been discussed.

Keywords. *Launaea pinnatifida* Cass; cytology; compositae.

1. Introduction

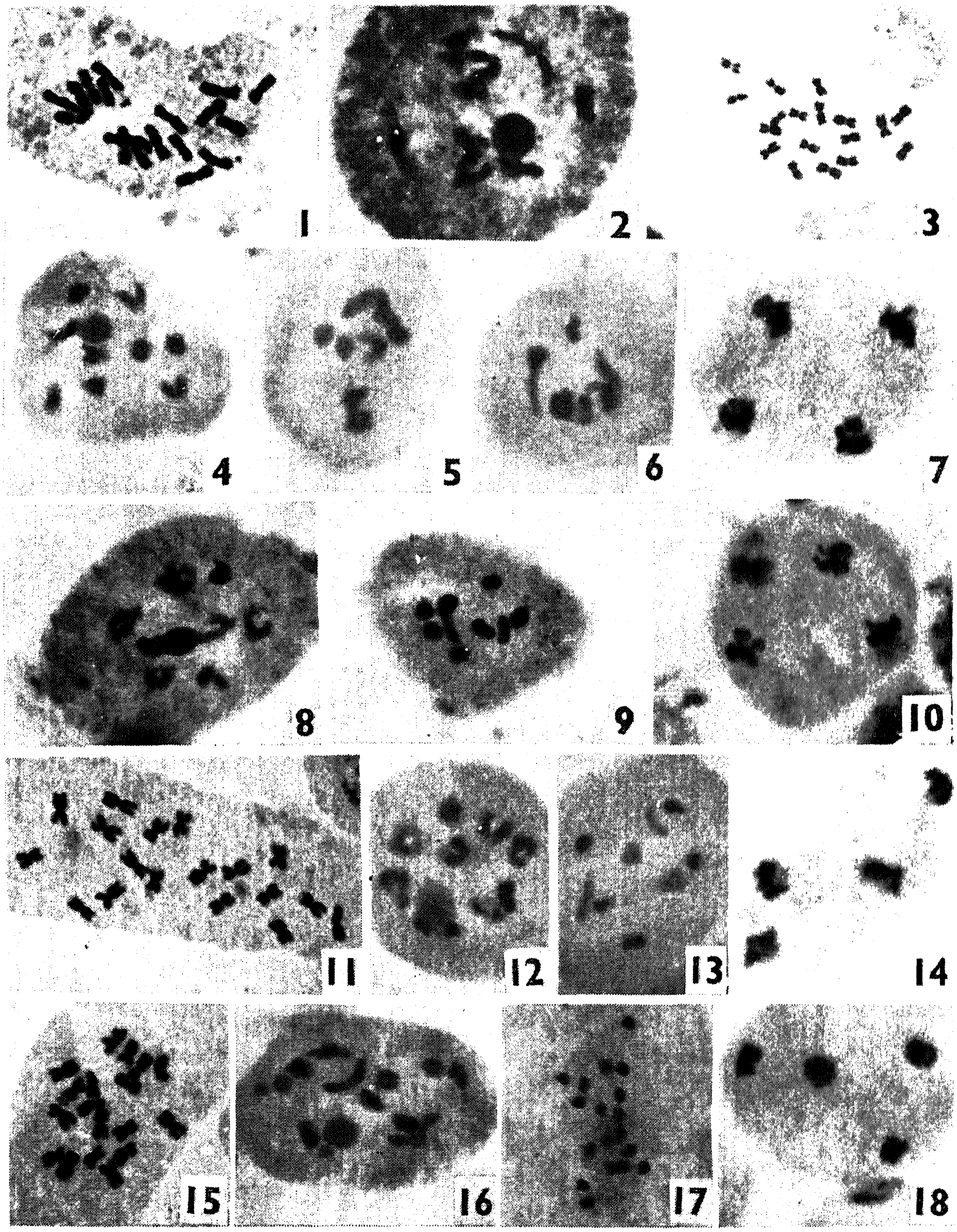
The compositae belongs to cosmopolitan family, extending from the Arctic to the sub-antarctic islands (but absent from the Antarctic continent) and from sea level to the snow line of the highest mountains (Solbrig 1969). *Launaea pinnatifida* Cass. belongs to the compositae and to the tribe cichorieae (Cooke 1958). The plant is a runner growing all along the sandy sea coasts of the Indian subcontinent. It produces milky latex and bears yellow flowered heads. Only chromosome counts are made for this plant (Chatterjee and Sharma 1969). Detailed cytological study, specially, with respect to its geographical distribution is felt necessary.

2. Materials and methods

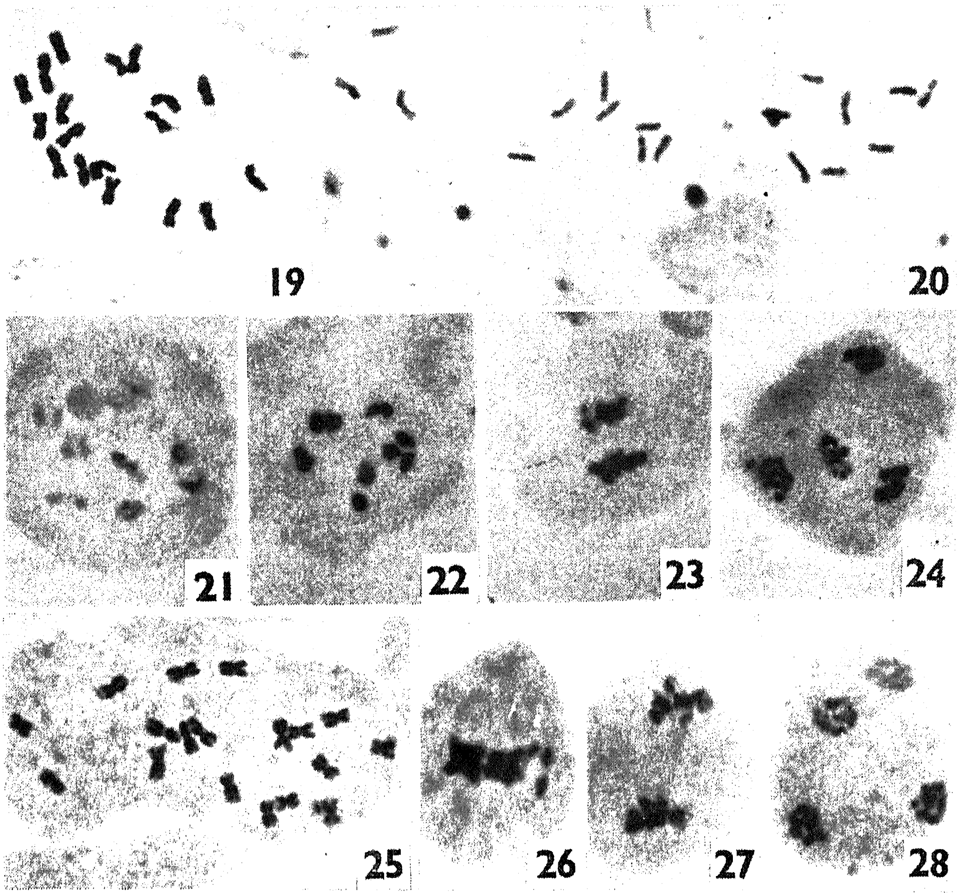
Plants were collected for the study during study tours to the following places—Ullal (near Mangalore), Merry Island (near Malpe), Kundapur, Honnavar, Karwar, Kumta, all these places are situated on sea coasts of Karnataka State; Madras, Pamban and other coastal places of Tamil Nadu and Pondicherry. Plants thus collected were grown in the departmental garden for the root-tip and flower-bud studies. Root-tips were treated with 0·002 M 8-hydroxy quinoline, squashed with aceto-orcein and further preparations were made as per aceto-orcein squash method (Tjio and Levan 1950). Flower buds of suitable size were fixed in acetic alcohol (1 : 3). The anthers were squashed in drop of 1% acetocarmine. The slides were made permanent by using butyl alcohol acetic acid series (Celariar 1959). While determining the centromere position the terminology given by Levan *et al* (1964) was adqpted.

3. Results and discussion

All the collections of *L. pinnatifida* showed $2n = 18$ chromosomes (figures 1, 3, 15, 19, 20 and 25). Coll. nos 120 and 77 showed all the 9 pairs with median region centromeres



Figures 1–18. **1.** Somatic metaphase plate of coll. no. 120 ($2n=18$). **2.** Diakinesis showing 9 II (coll. no. 68). **3.** Somatic metaphase plate (coll. no. 77) ($2n=18$). **4.** Diakinesis showing 9 II with one bivalent associated with nucleolus (coll. no. 77). **5.** Diakinesis showing 9 II (coll. no. 77). **6.** Diakinesis showing quadrivalents (coll. no. 77). **7.** Anaphase II, normal (coll. no. 77). **8.** Diakinesis showing 9 II with two bivalents associated with nucleolus (coll. no. 10). **9.** Metaphase-I showing 7 II and 1 IV (coll. no. 10). **10.** Anaphase II, normal (coll. no. 10). **11.** Somatic metaphase plate of coll. no. 122 ($2n=18$). **12.** Diakinesis showing 9 II (coll. no. 122). **13.** Metaphase-I, 9 II (coll. no. 122). **14.** Anaphase-II, normal (coll. no. 122). **15.** Somatic metaphase plate (coll. no. 214) ($2n=18$). **16.** Diakinesis showing 9 II (coll. no. 214). **17.** Anaphase-I, separation (coll. no. 214). **18.** Anaphase-II normal (coll. no. 214).



Figures 19–28. **19.** Somatic metaphase plate (coll. no. 110). **20.** Somatic metaphase plate (coll. no. 242) $2n = 18$. **21.** Diakinesis showing 9 II (coll. no. 242). **22.** Metaphase-I showing 9 II (coll. no. 242). **23.** Anaphase-I, normal (coll. no. 242). **24.** Anaphase-II, normal (coll. no. 242). **25.** Somatic metaphase plate (coll. no. 112) $2n = 18$. **26.** Metaphase-I (coll. no. 112). **27.** Anaphase-I (coll. no. 112). **28.** Tetrad formation (coll. no. 112).

(figures 1 and 3), while collection nos 110, 112, 122 and 242 have 8 pairs of median region centromeres and one pair with submedian centromere (figures 19, 25, 11, 20) respectively. And collection no. 214 has 7 pairs with median region centromeres and the remaining two pairs with submedian centromeres (figure 15). Further, there are slight variations with respect to range in length of chromosomes from place to place—the range is from 48–60 μm. This means cryptic changes leading to structural rearrangement or repatterning of chromosomes are going on within the taxon. 'Karyotype in the taxon at different localities show differences in the absolute chromosome size indicating change in nuclear DNA in evolution' (Mathew and Mathew 1983).

Meiosis is normal. Nine bivalents are counted at diakinesis. One or two nucleolar bivalents are noticed at diakinesis (figures 2, 4–10, 12–14, 21–24 and 26–28).

Coll. nos 77 and 10 showed quadrivalents and univalents at diakinesis and metaphase-I (figures 4 6, 8 and 9). The two genomes of the somatic complement have atleast two pairs of homologous chromosomes. The cause of low quadrivalent formation has been related to the paucity of pairing blocks, the position of the centromeres in the chromosomes and the chiasma interference across the centromere. There is also variation with regard to the number of nucleolar bivalents viz. one in collection no. 77 (figure 4) and two in collection no. 10 (figure 8). The taxon is less plastic regarding variation in chromosome numbers when compared to its allied taxon, *Launaea nudicaulis* Hook. with 2n = 18 and 36, (Solbrig 1977). It is the repatterning of the chromosomes that seemed to have played a major role in *L. pinnatifida*.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 5, October 1988, pp. 399 403.

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Seedling emergence and development of *Pinus kesiya* Royle ex Gord. and *Schima khasiana* Dyer

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Abstract. Early emergence was recorded in *Schima khasiana* as compared to *Pinus kesiya*. However, initial seedling growth was higher in the latter. Better yield was recorded during monsoon in both the species. While in *Pinus kesiya* shoot length increased up to the month of July and August, in *Schima khasiana* it continued up to September. The peak period for relative growth rate was recorded from August–September in *Pinus kesiya* and September–October in *Schima khasiana*. It was concluded that the conditions prevalent at the study site during August (temperature 23·5/17·5°C) and September (temperature 22·5/14°C) under high humidity (80–90%) and moderate rainfall (400 mm) may be appropriate for growth of transplants of *Pinus kesiya* and *Schima khasiana* respectively, under controlled conditions.

Keywords. *Pinus kesiya*; *Schima khasiana*; seedlings; relative growth rate.

1. Introduction

Seed germination and seedling growth of plants depend on interaction between their hereditary potentialities and the environmental conditions (Kramer and Kozlowski 1979; Kozlowski 1983; Minore 1986). Natural regeneration of many tree species is hindered by lack of suitable climatic conditions for seed germination and subsequent seedling establishment. Light, temperature and humidity are so interdependent that a change in one alters the others. The study of weather effects on emergence and seedling growth is important as it may provide an opportunity to produce large number of uniform transplants in controlled environment in a limited time (Kozlowski 1983).

Pinus kesiya Royle ex Gord. and *Schima khasiana* Dyer are two economically important early successional tree species which are predominant at high altitude in sub-tropics of north-east India. The emergence and seedling growth in relation to climatic factors during early phase of seedling establishment in both the species is poorly understood. Therefore, the present study was conducted to evaluate the influence of weather conditions during seedling development.

2. Materials and methods

Seedling emergence and their growth pattern in *P. kesiya* and *S. khasiana* were studied in relation to some climatic factors like temperature, humidity and rainfall at the University Campus, Shillong (latitude 25°35′ N and longitude 91°56′ E, altitude 1,500 m) during the year 1983–1984. The temperature, rainfall and humidity data were collected from the nearest meteorological centre in Shillong. The climate is divided by seasonal differences into (i) monsoonic season, with higher temperature, humidity and rainfall extending from May–October, (ii) dry and humid winter season from November–February followed by (iii) warm, dry summer of March and April (figure 1).

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Seeds of *P. kesiya* were collected from the University campus, Shillong (altitude 1,500 m) and those of *S. khasiana* from upper Shillong (altitude 1,800 m), Meghalaya, during the months of February–March 1983. The seeds of both the species were collected from many trees and then bulked separately. Fifty seeds of both the species were sown at about 1 cm soil depth in each plastic pots (30 cm diam) in open field conditions during April 1983. The duration of seedling emergence was recorded. Ten seedlings were maintained in each pot and 20 seedlings of each species in 4 groups of 5 were excavated every month. The root was washed thoroughly and length of root and shoot was measured. Seedlings were separated into root, shoot, needle/leaf and oven dried at 60 ± 5°C for 48 h. The increases in seasonal dry weight, length and relative growth rate (RGR) on dry weight basis of seedling and seedling parts were calculated and standard deviation computed.

3. Results

Seedlings emerged in *P. kesiya* and *S. khasiana* between 20–40 and 12–25 days, respectively, after sowing under open field conditions (table 1). The root and shoot elongations were more pronounced in *P. kesiya* as compared to *S. khasiana* at the time of seedling emergence. However, a reverse picture was obtained after a year of growth. On the other hand, root growth was significantly higher in *P. kesiya* after a year of growth. During the study of seasonal variation of climate, a better dry weight production of seedling and its parts in both the species (except for root) was observed in monsoon (table 2). The root dry weight was maximum during summer. As given in figure 2, *P. kesiya* seedlings showed maximum shoot elongation during July and August (mean temperature 23·5/17·5°C and RH 85–90%; average rainfall 400–

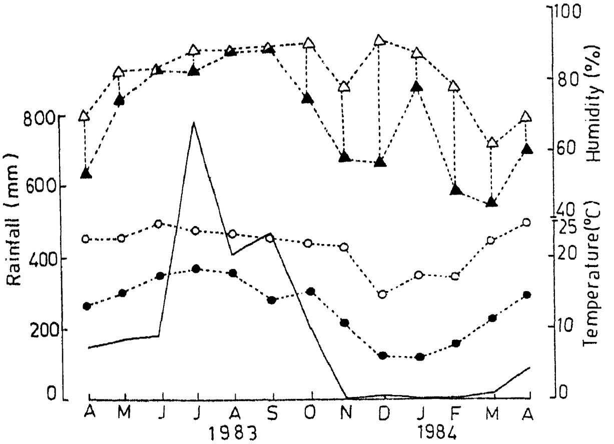

Figure 1. Climatic data of the study site (Shillong). Maximum temperature (○), minimum temperature (●), rainfall (———) and relative humidity (△, morning, ▲, evening).

Table 1. Emergence and growth of seedlings of *P. kesiya* and *S. khasiana* in field conditions.

	Growth (cm or cm²)/seedling part					
	After emergence			After one year		
Species	Root	Shoot	Needle/leaf	Root	Shoot	Needle/leaf
P. kesiya†	3·00 ± 0·10	4·35 ± 0·20	2·45 ± 2·02	32·80 ± 0·10	11·50 ± 0·10	3·60 ± 0·25
*S. khasiana**††	2·10 ± 0·10	3·00 ± 0·05	0·70 ± 0·02	19·80 ± 0·01	15·50 ± 1·90	9·10 ± 1·55

Emergence period: days of initiation of germination, †20, ††12; days of final germination, †40, ††25.
*Leaf (cm^2)
± SD

Table 2. Seasonal variations in yield of *P. kesiya* and *S. khasiana* seedlings during the course of one year.

	Yield (dry wt in mg/seedling part)		
	Seasons		
Seedling part	Monsoon	Winter	Summer
P. kesiya			
Seedling	371·50 ± 25·70	5·10 ± 0·50	103·40 ± 24·00
Shoot	317·90 ± 26·30	–	26·60 ± 3·80
Root	53·60 ± 0·60	37·60 ± 7·20	76·80 ± 4·90
Needle	1·60 ± 0·10	–	1·35 ± 1·20
S. khasiana			
Seedling	560·00 ± 16·40	–	503·00 ± 28·50
Shoot	465·60 ± 25·20	–	284·00 ± 12·60
Root	94·20 ± 11·60	102·80 ± 9·25	219·00 ± 21·00
Leaf	42·50 ± 0·70	–	13·10 ± 1·50

± SD
–, No increase in growth.

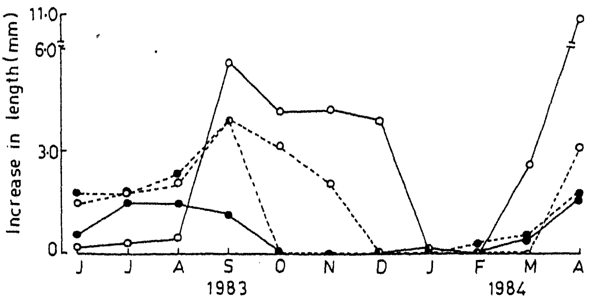

Figure 2. Increase in root (○) and shoot (●) elongations during the first year development of *S. khasiana* (- - - - -) and *P. kesiya* (————) seedlings.

800 mm, figure 1) which was almost negligible during winter (mean temperature 17·5/7·5°C and RH 60–85%; no rainfall). The maximum increase in root elongation in this species was recorded during September (mean temperature 22·5/14°C and RH 85–90%, average rainfall 450 mm) and was completely inhibited during

January–February (mean temperature 17·5/7·5°C and RH 65–80%; no rainfall). The maximum increase in shoot elongation of *S. khasiana* was recorded during September and thereafter it was markedly inhibited at the beginning of winter (figure 2). The recurrence of its growth was observed at the onset of summer. The root growth was also maximum in September and subsequently it declined gradually. It was inhibited completely from December–March and thereafter it resumed the growth. The peak periods of RGR in *P. kesiya* and *S. khasiana* seedlings were found in August–September (mean temperature 22·5/15°C and RH 80–90%; average rainfall 400–500 mm) and September–October (mean temperature 22·5/15°C and RH 80–90%; average rainfall 200–450 mm), respectively (table 3). The negative RGR were obtained in shoot of both the species during October–December (mean temperature 15/10°C and RH 60–85; rainfall 0–200 mm), in *P. kesiya* needles during October–February and *S. khasiana* leaf during October–January. The seedlings also showed negative RGR from October–December and November–December in *P. kesiya* and *S. khasiana*, respectively.

4. Discussion

The early seedling emergence in *S. khasiana* was recorded as compared to *P. kesiya*. The probable cause of early germination in the former is due to high permeability of the seed coat (Mayer and Poljakoff-Mayber 1982). Seed size (Silvertown 1981) and seed mass (Thomas 1966) have important ecological consequences in seedling vigour. During the present study of seedling emergence, the higher seed mass in *P. kesiya* when compared to that in *S. khasiana* may bear a direct correlation with seedling elongation. However, a reverse picture obtained for the two species after a year is due to difference in their genetic make up. Similar conclusions have been drawn with regard to seed weight and dry weight of one year old seedlings of 6 tree species grown in Mediterranean forest of western Australia (Abbott 1984). The maximum dry weight production of seedling and its above parts in both *P. kesiya* and *S. khasiana* during monsoon was directly related to optimum conditions of temperature, rainfall and humidity required for growth. But root dry weight was

Table 3. Mean relative growth rate (mg mg^{-1} day^{-1}) of *P. kesiya* and *S. khasiana* seedlings during the course of one year.

	P. kesiya				*S. khasiana*			
Months	Seedling	Shoot	Root	Needle	Seedling	Shoot	Root	Leaf
M–J	0·508	0·482	0·026	0·003	0·774	0·598	0·108	0·092
J–J	0·879	0·817	0·062	0·002	1·383	1·172	0·211	0·120
J–A	1·231	1·164	0·067	0·002	1·763	1·449	0·314	0·175
A–S	8·500	7·155	1·345	0·021	3·463	3·097	0·516	0·280
S–O	0·584	0·387	0·197	0·000	10·735	8·812	1·923	0·790
O–N	−0·367	−0·549	0·082	−0·007	0·155	−1·168	1·323	−0·005
N–D	−0·141	−0·151	0·010	−0·001	−1·130	−2·226	1·095	−0·021
D–J	1·908	1·081	0·829	−0·002	0·436	0·353	0·084	−0·059
J–F	0·544	0·254	0·290	−0·014	1·134	0·541	0·593	0·053
F–M	0·605	0·376	0·230	0·007	3·005	2·512	1·493	0·080
M–A	2·768	0·504	2·265	0·073	7·744	3·996	3·729	0·426

maximum during summer on account of higher development of roots needed for increased absorption (Mason *et al* 1970). Hence, it could be concluded that exponential growth of plants is time dependent and environmentally controlled (Ågren 1985).

The shoot elongation of *P. kesiya* and *S. khasiana* were maximum during July–August (temperature 23·5/17·5°C and RH 85–90%; rainfall 400–800 mm) and September (temperature 22·5/14°C and RH 85–90%; rainfall 450 mm), respectively. Both root and shoot were highly sensitive to low temperature (corresponding to 17·5/7·5°C of day and night) of winter. Besides, RGR revealed that August–September and September–October were optimum periods for the seedling growth in *P. kesiya* and *S. khasiana*, respectively. The negative RGR of the seedling and its above parts in both the species was due to needle and leaf fall during winter. The comparative study of increase in length and RGR of various parts exhibited that prevailing conditions in August (temperature 23·5/17·5°C, RH 85–90%; rainfall 400 mm) and September (temperature 22·5/14°C; RH 85–90; rainfall 450 mm) may be optimum for production of nursery transplants of *P. kesiya* and *S. khasiana*, respectively. The occurrence in field conditions of alternating temperatures of 23·5/17·5°C for *P. kesiya* and 22·5/14°C for *S. khasiana* resulted in higher growth under high humidity (Approx. 80–90%) and moderate rainfall (Approx. 400 mm) exhibiting the thermoperiodic behaviour of growth in both the species.

Acknowledgement

This study was supported by a research grant to PT from Indian National Science Academy, New Delhi.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 5, October 1988, pp. 405–407.
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Yield and oil content of doubled haploids of mustard (*Brassica juncea* (L.) Czern. and Coss.)

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Abstract. Progenies of androgenetic doubled haploid plants of mustard cv TM-4 in A_4–A_6 generations were evaluated for their seed yield and oil content. Eight progenies in the A_5 generation out of the 97(8%) A_1 plants regenerated were equal in yield to the parent cultivar. The oil percentage in these doubled haploids were in the same range as that of the parent cultivar. The remaining androgenetic doubled haploids were statistically lower in yield. The data indicates that about 8% of the *in vitro* raised androgenetic plant progenies had no adverse effects on yield potential and point to the need for obtaining a large number of androgenetic doubled haploids to recover genotypes more productive than the better parent.

Keywords. *Brassica juncea*; Indian mustard; doubled haploids; yield; oil content.

1. Introduction

Wide occurrence of *in vitro* cell culture induced variation (Scowcroft and Larkin 1983; Evans *et al* 1984) is likely to adversely affect the yield potential of the *in vitro* raised plant progenies in subsequent generations. Therefore yield evaluation of the progenies of the *in vitro* raised plants is necessary for using the anther culture technique in plant breeding programmes. Probability of recovering genotypes without any adverse effect of *in vitro* induced genetic variation on yield can help in arriving at reliable estimates of the total effort required in a breeding programme. The objective of the present study was to evaluate the yield potential and oil content of androgenetic doubled haploids of mustard cv TM-4 reported earlier (George and Rao 1982) in comparison to the parent cultivar.

2. Materials and methods

Among the 15 cultures used (Rai-5, Varuna, T-59, TM-1, TM-2, TM-3, TM-4, TM-5, TM-6, TM-7, TM-8, TM-9, TM-11, TM-12 and TM-13) in anther culture, only TM-4 showed some positive response. The *in vitro* culture methods used have been described previously (George and Rao 1982). Single plants were carried forward as progeny lines. The first generation androgenetic plants were designated as A_1 and the subsequent progenies of these plants were designated A_2, A_3 etc. A_2 and A_3 plants were raised in pots. A_4–A_6 plant progenies were evaluated for yield as in a practical breeding programme from the year 1984–1987 *rabi* seasons. The lines yielding lower than the parent TM-4 were discarded each year, thus 44, 17 and 8 progenies respectively were evaluated in A_4–A_6 generations.

Yield trials were conducted in randomised block design with 2, 4 and 7 replications during 1984–1985, 1985–1986 and 1986–1987 respectively. Each plot ($3{\cdot}6\ m^2$)

Table 1. Yield (g/m^2) of androgenetic doubled haploid plants of TM-4 mustard in A_4–A_6 generations.

Androgenetic progeny	1984–1985 (A_4)	1985–1986 (A_5)	1986–1987 (A_6)	1986–1987 (oil%)
TM-4(Parent)	67	79	121	31·5
A14-82	92	76	108	33·5
A15-83	96	82	113	32·2
A16-82	104	74	121	32·2
A18-82	88	76	117	32·9
A19-82	92	86	96	31·4
A24-82	104	67	108	31·4
A25-82	101	56	—	—
A26-82	112	73	117	31·3
A26-83	90	59	—	—
A28-82	93	63	—	—
A31-83	103	76	121	33·0
A35-83	97	59	—	—
A47-83	120	49	—	—
LSD (at 5%)	23·4	9·9	25·0	0·68

comprised of 3 rows, 4 metre long with 30 cm spacing between rows and 10 cm between plants. Urea (50 kg N/ha) was applied 20 days after sowing. The crop was irrigated and pests were controlled with 0·07% malathion or demacron sprays as and when required. Oil percentage in the seeds harvested from 1986–1987 experiment was estimated using nuclear magnetic resonance (NMR) technique as described previously (Srinivasan *et al* 1985).

3. Results and discussion

Yield and oil per cent of androgenetic doubled haploid progenies tested in the A_4–A_6 generations are given in table 1. Out of 97 A_1 plants and after elimination of lower yielding progenies, 8 lines were statistically equal in yield to the parent. These lines also did not show any appreciable difference in oil percentage. Reduced yield potential of the *in vitro* regenerated plant progenies in other crops have been reported previously (De Paepe *et al* 1981; Schnell and Wernsman 1986). In barley, doubled haploid families from majority of crosses were inferior to mid parent but some were superior to the better parent (Friedt *et al* 1986). Though there are several reports on the development of new, more productive cultivars using anther culture (De Buyser *et al* 1987), the total *in vitro* effort which resulted in such new cultivars is often not indicated in the publications. De Buyser *et al* (1987) reported development of new wheat cultivar 'Florin' from 41 doubled haploids obtained after planting 49266 anthers of the F_1 plants. In the present study 8 out of 97 (8·2%) androgenetic plants were equal in yield to the parental cultivar and the remaining were inferior. The results point to the need of obtaining androgenetic doubled haploids in large numbers to recover genotypes more productive than the better parent.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 5, October 1988, pp. 409–417.
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Foliar epidermis and taxonomy in Apocynaceae

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MS received 24 June 1987; revised 20 June 1988

Abstract. Mature epidermis and stomatal ontogeny were studied in the leaves of 10 species (9 genera) belonging to Apocynaceae. Abaxial intercostal cells of *Wrightia tinctoria* are straight-walled and those of adaxial side in *Wrightia tinctoria*, *Cascabela thevetia* and *Carissa spinarum* are sinuous. Adaxial epidermis of *Rauvolfia tetraphylla* is papillose. Hairs are thin-walled in *Plumeria rubra* (unicellular) and *Carissa spinarum* (uniseriate) and thick-walled in *Rauvolfia tetraphylla*, *Catharanthus roseus* (uni- and multicellular) and *Catharanthus pusillus* (unicellular). Leaves of *Wrightia tinctoria*, *Ervatamia divaricata* and *Catharanthus* spp. are amphistomatic while those of other species are hypostomatic. Four stomatal types viz. anomo-, aniso-, tetra- and paracytic, occur in different combination patterns. Codominance of two stomatal types and their origin from unilabrate meristemoids isolate *Catharanthus* from other taxa. Its primitive nature is indicated by the unilabrate meristemoids.

Keywords. Apocynaceae; foliar epidermis; stomatal ontogeny; taxonomic significance.

1. Introduction

A number of papers have been published on the epidermal features of several members of Apocynaceae. Metcalfe and Chalk (1950) summarised the earlier works on the leaf epidermis. Chandra *et al* (1969) and Kapoor *et al* (1969) studied the epidermal characters and venation patterns in many species, bringing out their identificatory value. Trivedi and Upadhyay (1973, 1974) reported the stomatal and trichome morphology of *Rauvolfia serpentina* and the occurrence of giant stomata in several taxa. Sud (1984) brought out the stomatal abnormalities in the leaves of *Catharanthus roseus*. Based on numerical taxonomic studies, Balamani and Rao (1984) clarified the ambiguities in the classification of Schumann (1895). However, there appears to be no report on the ontogeny of stomata in this family. The present study is on the foliar epidermis and stomatogenesis in 10 species and their taxonomic importance.

2. Materials and methods

Leaves of different ages were collected from 10 species belonging to 9 genera (table 1) growing in and around JIPMER Campus and fixed in FAA. Epidermal peels were taken from the fresh leaves as well as fixed materials mechanically. Leaf bits (1 × 0·5 cm) were also cut from the basal, middle and apical regions of leaf and cleared in NaOH solution and chloral hydrate successively. These leaf bits were washed in water and stained with aqueous safranin (1%) or Delafield hematoxylin. Stained materials were mounted in glycerine (50%) and sealed with DPX.

Table 1. List of species studied and their taxonomic position.

Species	Balamani and Rao (1984)	Schumann (1895)
Vallaris solanacea (Roth.) Kuntze. *Wrightia tinctoria* (Roxb.) R. Br.	Sub-family Echitoideae (Cluster I)	Sub-family Echitoideae
Carissa spinarum Linn. *Rauvolfia tetraphylla* Linn. (*R. canascens* Linn.)	Sub-family Plumieroideae Tribe: Carisseae (Cluster II)	Sub-family Plumieroideae Tribe: Plumiereae
Ervatamia divaricata (L.) Burkill (*Tabernaemontana coronaria* (Jacq.) Willd.	–do–	–do–
Plumeria rubra Linn.	Tribe: Plumiereae (Cluster III)	–do–
Allamanda cathartica Linn. *Cascabela thevetia* (L.) Lippold. (*Thevetia neriifolia* Juss. and Steudol)	Tribe: Allamandeae (Cluster IV)	–do–
Catharanthus roseus (L.) Don. (*Vinca rosea* L.) *Catharanthus pusillus* (Murray) (*Vinca pusilla* Murray) Don.	Tribe: Catharantheae	–do–

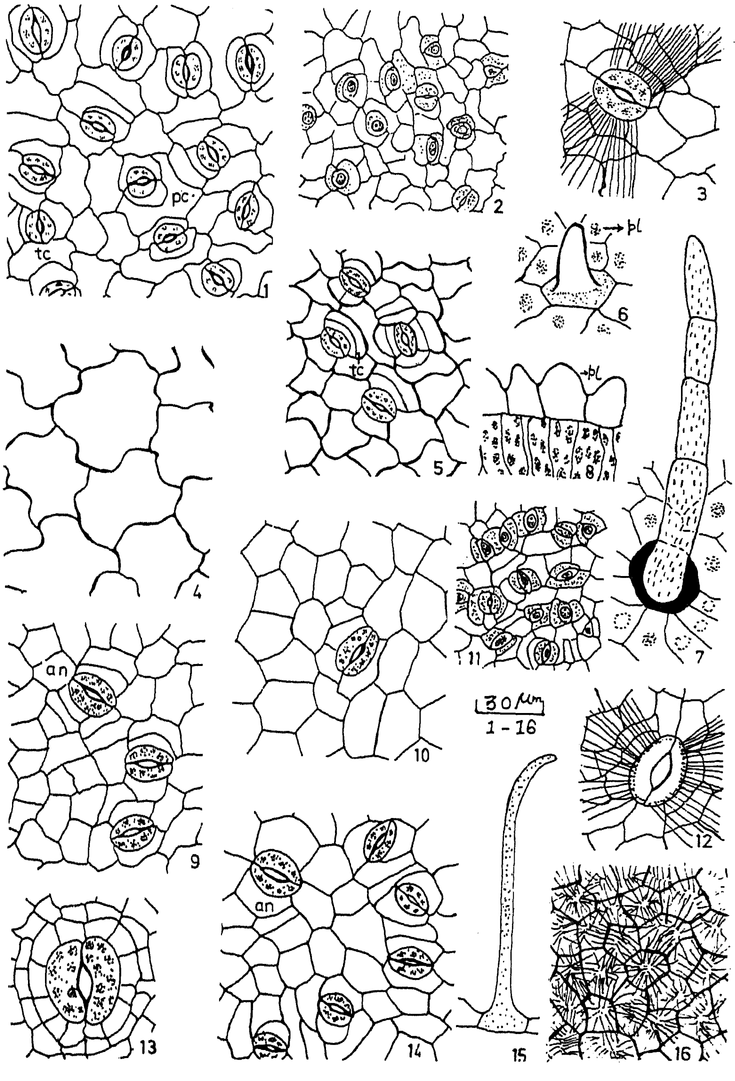
Transections were employed wherever necessary to observe the morphology of hairs. Names of the species were adopted from the Flora of the Tamil Nadu Carnatic (Matthew 1983).

3. Results

3.1 *Mature foliar epidermis*

3.1a *Abaxial surface*: The costal cells in all species studied, are usually elongated, rectangular to rhomboidal, thin-walled; however, those of *Wrightia tinctoria* and *Carissa spinarum* are thick-walled. Intercostal cells are invariably isodiametric, polygonal or sometimes irregular-shaped. All species except *C. spinarum* (unevenly thick-walled) possess thin-walled cells. Exceptionally the intercostal cells of *W. tinctoria* are straight-walled. They are arched in *Vallaris solanacea* (figure 1), *Allamanda cathartica* and *Plumeria rubra* (figure 14); slightly sinuous in *Ervatamia divaricata* (figure 9) and *Rauvolfia tetraphylla*; sinuous in *Cascabela thevetia*; deeply sinuous in *Catharanthus* spp. (figures 21, 30). Some costal cells and intercostal cells in *R. tetraphylla* tend to be papillose. Cuticular striations are long and slightly undulated in *R. tetraphylla*, *A. cathartica* and *W. tinctoria*. But leaves of *P. rubra* show short and reticulate striations arising from the central part of the epidermal cells (figure 16).

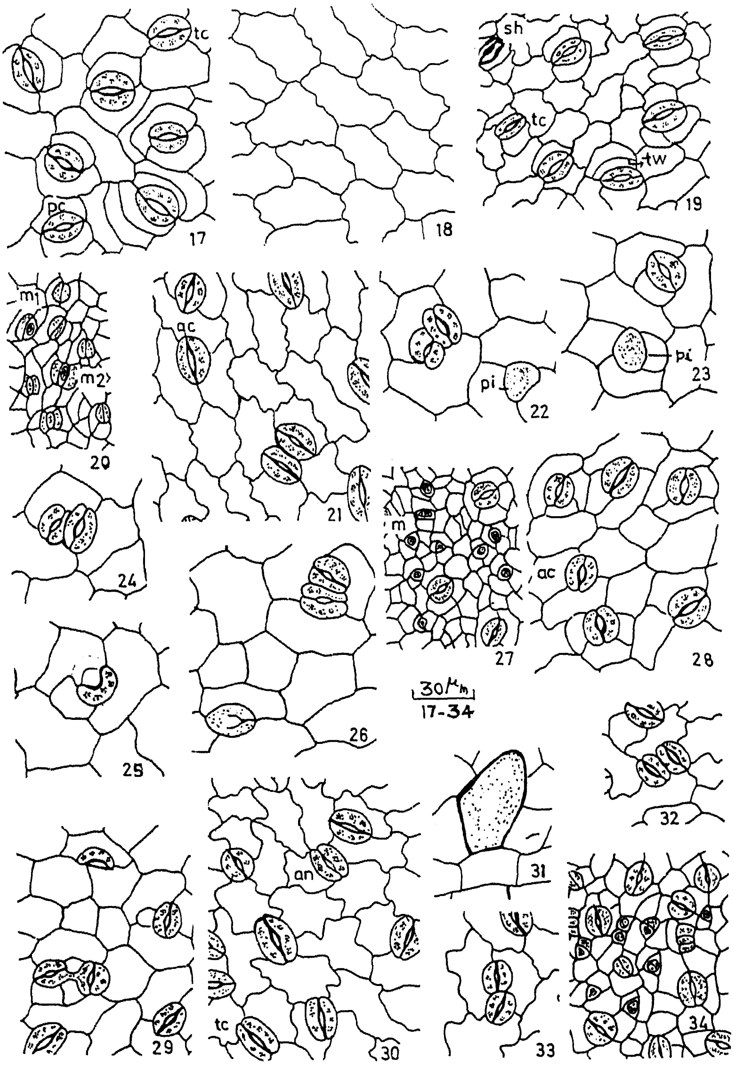
Four stomatal types viz., tetra-, aniso-, para- and anomocytic are encountered in the species studied and their distribution is given in table 2. One or two types are absent in some species. All species show tetracytic stomata (figures 1, 5, 9, 17, 19, 21) in different frequencies. Anisocytic type is seen in 8 species except *C. thevetia* and *P. rubra* (figures 1, 21, 28); paracytic ones in 7 species (figures 1, 14); anomocytic stomata in 7 species (figures 9, 21, 30).



Figures 1–16. 1–7, 9–16. Leaf epidermis (surface view) **1 and 2.** *V. solanacea.* **1.** Abaxial. **2.** Abaxial-developmental stages of stomata. **3.** *W. tinctoria*—adaxial. **4 and 5.** *C. spinarum.* **4.** Adaxial. **5.** Abaxial. **6–8.** *R. tetraphylla.* **6.** Unicellular hair. **7.** Uniseriate hair. **8.** Ts showing papillae on adaxial surface. **9–11.** *E. divaricata.* **9.** Abaxial. **10.** Adaxial. **11.** Developmental stages of stomata. **12–16.** *P. rubra.* **12.** Abaxial; note giant stoma and cuticular striations. **13.** Abaxial of young leaf showing giant stoma. **14.** Abaxial. **15.** Unicellular hair (abaxial). **16.** Adaxial.

Giant stomata are usually one and a half times larger than normal ones. They are frequently seen in most of the species. One or two stomata of very large size are very rarely observed among the smaller differentiating protodermal cells of young leaves of *P. rubra* (figure 13).

Stomatal abnormalities observed in the species studied include shrivelled stomata with thickened pore (figure 19) (*C. thevetia*), contiguous stomata (superposed,



Figures 17–34. Leaf epidermis (surface view). **17.** *A. cathartica*—abaxial. **18–20.** *C. thevetia*. **18.** Adaxial. **19 and 20.** Abaxial. **19.** Mature leaf. **20.** Developing stomata. **21–27.** *C. roseus*. **21.** Abaxial. **22–26.** Adaxial—stomatal abnormalities. **27.** Abaxial—developing stomata. **28–34.** *C. pusillus*. **28 and 29.** Adaxial. **30.** Abaxial. **31.** Adaxial—unicellular hair in costal region. **32 and 33.** Contiguous stomata. **34.** Abaxial—developing stomata.
(ac, Anisocytic stoma; an, anomocytic stoma; dc, degenerating cell; m, meristemoid; m1, first mesogenous subsidiary cell; m2, second mesogenous subsidiary cell; pc, paracytic stoma; pi, persistent stomatal initial; pl, papilla; sc, sister cell; sh, shrivelled stoma; tc, tetracytic stoma; tw, supplementary tangential wall).

juxtaposed and oblique) of equal and unequal guard cells (figures 21, 22, 24, 32, 33), stomata with one or two degenerated guard cells (figures 25, 26, 29), stomata with plasmodesmata connection (figure 29) and persistent stomatal initials (figures 22, 23) (*Catharanthus* spp.).

Table 2. Stomatal features in Apocynaceae.

						Stomata/size of stomata				
			Stomatal types (%)				Normal		Giant (μm)	
Name of species	Surface	Anomo-	Aniso-	Para-	Tetra-	mm^2	L	B	L	B
Vallaris solanacea	Abaxial	Rare	22	68	10	600	22	15·5	30	19
	Adaxial	—	—	—	—	—	—	—	—	—
Wrightia tinctoria	Abaxial	—	20	74	6	390	27	19	28	19
	Adaxial	—	25	70	5	30	34	24	36	25
Carissa spinarum	Abaxial	15	4	Rare	81	360	22	15·5	—	—
	Adaxial	—	—	—	—	—	—	—	—	—
Rauvolfia tetraphylla	Abaxial	19	4	4	73	345	25	18	36	21
	Adaxial	—	—	—	—	—	—	—	—	—
Ervatamia divaricata	Abaxial	15	3	—	82	354	31	22	34	25
	Adaxial	—	—	—	Very few	11	31	22	34	25
Plumeria rubra	Abaxial	—	—	20	80	555	27	17	37	26
	Adaxial	—	—	—	—	—	—	—	—	—
Allamanda cathartica	Abaxial	—	4	80	16	690	25	18	28	19
	Adaxial	—	—	—	—	—	—	—	—	—
Cascabela thevetia	Abaxial	14	—	5	81	525	27	16	—	—
	Adaxial	—	—	—	—	—	—	—	—	—
Catharanthus roseus	Abaxial	6	40	—	54	411	27	22	34	25
	Adaxial	8	42	—	50	145	27	22	33	25
Catharanthus pusillus	Abaxial	35	15	—	50	642	27	19	36	25
	Adaxial	32	19	—	49	341	28	21	36	25

—, Absent; L, length; B, breadth.

Only non-glandular hairs are seen in the following 5 species. Thin-walled hairs are either unicellular (confined to costal and margins in *P. rubra* figure 15) or uniseriate (costal and intercostal regions of young leaves of *C. spinarum*). Unicellular hairs are rarely seen in *C. pusillus* (figure 31). Uniseriate warty hairs and unicellular hairs occur frequently in *R. tetraphylla* and *Catharanthus roseus.*

3.1b *Adaxial surface*: The costal cells are almost similar to those of the abaxial epidermis. Intercostal cells are usually isodiametric, polygonal; walls thin, straight or arched (figures 10, 16, 26, 28, 29). But cell walls in *C. spinarum* (figure 4), *C. thevetia* (figure 18) and *W. tinctoria* (figure 3) are sinuous. Most of the costal and intercostal cells in *R. tetraphylla* are prominently papillose (figure 8), as seen in transection. In surface view they appear as circular structures in the centre of the cells (figures 6, 7). Cuticular striations (figures 3, 16), hairs, stomatal types and stomatal abnormalities are similar to those of abaxial surface.

3.2 *Stomatogenesis*

Three types of meristemoids are encountered in the present study. They are alabrate (without any cutting face), unilabrate (with one cutting face) and dolabrate (with two cutting faces). Each type gives rise to one or more than one adult stomatal type.

3.2a *Alabrate type*: Meristemoids of this type are observed in the leaves of *V. solanacea*, *R. tetraphylla*, *P. rubra* and *Catharanthus* spp. and always arise much earlier compared to those of other types. The meristemoid acts as guard cell mother cell (GMC) directly and divides equally to produce two guard cells. Hence these stomata are of perigenous type (figures 3, 12, 21, 30).

3.2b *Unilabrate type*: This is seen only in *Catharanthus* spp. The meristemoid divides unequally to produce one large mesogenous subsidiary cell and small GMC. The latter gives rise to a pair of guard cells (figures 27, 34). Depending upon the number of perigenous subsidiaries encircling the guard cells (i.e. 2, 3, 4) the stoma becomes aniso-, tetra- and anomocytic.

3.2c *Dolabrate type*: This type is encountered in all the species except *Catharanthus* spp. The meristemoid cuts off two subsidiaries and a GMC by two unequal divisions (figures 2, 11, 20). Three adult stomatal types result depending upon the intersection of the second wall with the first one (Kannabiran 1975). (i) Para-mesogenous type develops when the second wall intersects the first one at both the poles (figure 2). (ii) Aniso-mesoperigenous type is derived when the second wall intersects the first one at only one pole (figure 11). (iii) Tetra-mesoperigenous stoma is formed when the second wall lies parallel to the first one without intersecting the former (figures 11, 20).

In some species (*C. thevetia*, *C. spinarum*, *E. divaricata* and *A. cathartica*) mesogenous subsidiaries sometimes divide once tangentially to produce a pair of lateral subsidiaries on either side (figures 5, 17, 19). In *R. tetraphylla* and *C. spinarum* one or two subsidiaries may divide radially to increase their number (figure 5).

Contiguous stomata in *Catharanthus* spp. arise by the formation of adjacent meristemoids (figure 34). In case they differentiate simultaneously, they are of similar size; otherwise they are of dissimilar size (figures 22, 24, 26).

4. Discussion

Intercostal cells of abaxial and adaxial epidermis are sinuous and straight-walled in most of the species respectively. But those of abaxial surface in *W. tinctoria* are straight-walled while those of adaxial side in *W. tinctoria*, *C. spinarum* and *C. thevetia* are sinuous. Such features (sinuous adaxial cells and straight-walled abaxial cells) have been reported to be of rare occurrence and observed in only one species (*Reevesia wallichii*) out of 66 species of Malvales (Shanmukha Rao and Ramayya 1983a). Papillose nature of adaxial epidermis in the leaves of *R. tetraphylla* is brought out for the first time though the same was described in other species earlier (Metcalfe and Chalk 1950). Cuticular striations are usually long and they originate from the guard cells. But, in *P. rubra* these are short and reticulate arising from the central part of the epidermal cells.

The present study confirms the earlier observations on stomatal abnormalities in *Cascabela* and *Catharanthus* spp. (Kapoor *et al* 1969; Sud 1984). Though the giant stomata are seen in most of the species, giant stomata of such large size, reported by Trivedi and Upadhyay (1973) could not be observed even in *R. tetraphylla*. The giant stomata are always derived from the alabrate meristemoids much earlier than normal ones.

Predominance of a particular type was explained as a reflection of the precarious balance between the influences operating at the level of the meristemoid that tend to cause the formation of stomatal mother cells on one hand and the influences operating at the level of organ involved in the orientation of the cell divisions on the other hand (Guyot *et al* 1968; Humbert and Guyot 1969). Hence variations in the stomata on the same organ could thus be correlated with the phylogenetic status, especially when we consider the predominance of a particular stomatal type in the taxon concerned (Guyot 1971). The above views are substantiated by the present observations. The sub-family Echitoideae is characterised by the predominance of para-mesogenous stomata and indicates its advanced nature also (Kannabiran and Krishnamurthy 1979). The tribes Carisseae and Plumiereae show tetra-mesoperigenous type predominantly. However, Allamandeae appears to be heterogeneous because each genus is characterised by the predominance of either para-mesogenous or tetra-mesoperigenous type. Catharantheae is distinguished by the co-dominance of two types (tetra- and aniso-mesoperigenous or tetra- and anomo-mesoperigenous types) occurring almost in the same frequency (Shanmukha Rao and Ramayya 1983b).

Evidences from the stomatal morphology and ontogeny broadly support the classification of Schumann (1895) as altered by Balamani and Rao (1984) based on numerical taxonomic studies; however, they suggest some modifications also. Based on the analysis of 84 characters in 25 species the above authors proposed 5 clusters.

Cluster I comprises of the members of sub-family, Echitoideae. This was considered to be primitive supporting the work of Woodson (1930). But stomatographic studies reveal its advanced nature by the predominance of para-mesogenous stomata in the species studied presently and previously (Chandra *et al* 1969; Kapoor *et al* 1969). These para-mesogenous stomata were shown to be the advanced type by the earlier workers during their studies in Umbelliferae, Papilionaceae and Cornales and Umbellales (Kannabiran and Krishnamurthy 1979).

Cluster II is the tribe Carisseae with two sub-tribes, Rauvolfinae (*C. spinarum* and *R. tetraphylla*) and Alstoninae (*E. divaricata*). This tribe, as a whole is distinguished by the predominance of tetra-mesoperigenous stomata. The former sub-tribe is distinguished by hypostomatic leaves with hairs and the latter by amphistomatic leaves devoid of hairs.

The placement of *Plumeria* under the tribe Plumiereae (cluster III) and its isolation from other genera such as *Tabernaemontana* (*Ervatamia*), *Alstonia*, *Holarrhena* and *Catharanthus* is also justified by the evidences from cytological, wood anatomical and embryological studies. This view is further supported by the presence of only para- and tetracytic stomata in this genus.

Cluster IV consists of the tribe Allamandeae in which two genera, *Allamanda* and *Cascabela* are studied. These taxa are closely related by all morphological characters but differ in habit, phyllotaxy, placentation and fruit characters. While the dolabrate nature of the meristemoid unites them, adult stomatal type separates them. *Allamanda* shows tetracytic type predominantly whereas *Cascabela* exhibits paracytic type frequently.

Treatment of *Catharanthus* under a tribe of its own, Catharantheae (cluster V) is supported by the co-dominance of two stomatal types (tetra- and anisocytic in *C. roseus* and tetra- and anomocytic in *C. pusillus*) and the development of these stomata from unilabrate meristemoids. The latter feature also indicates its primitive nature compared to those with dolabrate meristemoids (Kannabiran and Krishnamurthy 1979).

Acknowledgements

The authors express their deep gratitude to Dr K Venkatasubramanian, Vice-Chancellor, Pondicherry University, Pondicherry for his encouragement. One of the authors (VR) thanks Prof. P K Chidambaram, Principal, Arignar Anna Government Arts College, Karaikal for the facilities.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 5, October 1988, pp. 419–424.
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Effect of gibberellic acid on nitrate utilization in *Sesamum indicum* seedlings

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MS received 2 September 1987; revised 20 June 1988

Abstract. Effect of gibberellic acid was studied on nitrate utilization in *Sesamum* seedlings grown under continuous light in standard mineral medium. Gibberellic acid treatment markedly inhibited nitrate uptake and its reduction. Sucrose 2% (w/v), when added in the incubation medium, significantly reversed this inhibition. Further, sugars and chlorophyll contents were also low and Hill-reaction activity recorded inhibition in GA_3 treated seedlings. The poor uptake and reduction of nitrate is a result of low level of tissue sugars in gibberellic acid treated seedlings.

Keywords. Nitrate uptake; nitrate reduction; nitrate reductase activity; total sugars; Hill-reaction activity; chlorophyll; gibberellic acid.

1. Introduction

Nitrate (NO_3^-) is usually the main source of N for many crop plants including legumes (Neyra *et al* 1977; Ryle *et al* 1978). NO_3^- utilization in higher plants is essentially the integration of uptake and reduction of NO_3^- and transfer of NO_3^- or reduced NO_3^- to other cells and tissues (Shaner and Boyer 1976). Both uptake and reduction of NO_3^- are substrate inducible processes (Breteler *et al* 1979). NO_3^- reductase (EC 1·6·6·1) is the first enzyme in the NO_3^- reduction pathway and play critical role in NO_3^- utilization (Naik *et al* 1982).

Nitrate reductase (NR) level in higher plants have been shown to fluctuate in response to several factors like change in environmental conditions (Guerrero *et al* 1981), metabolic factors like protein synthesis (Jackson *et al* 1974), carbohydrate supply (Aslam and Oaks 1975; Zink 1982) and reduced N (Stewart 1972). Some reports have also appeared on the regulation of nitrate reductase by various phytohormones (Knypl 1979), however, marked contradiction in results indicate that the effect of phytohormones may not be directly on NR induction but on various factors controlling nitrate reductase activity (NRA). Induction of NR in plants is a dynamic interaction with NO_3^- uptake, accumulation and reduction (Aslam *et al* 1987) hence mere estimation of NRA is unlikely to reveal any controlling mechanism of phytohormones on NO_3^- utilization. Gibberellic acid (GA_3) is most extensively studied phytohormone for its effect on NRA and have been reported both to promote (Knypl 1979) as well as to inhibit NRA (Knypl and Janas 1979). In the present communication GA_3 and its effects were studied on various factors determining NO_3^- utilization in *Sesamum* seedlings.

2. Materials and methods

Seeds of *Sesamum indicum* L. CV Gujarat Tel 1 were surface sterilized with 0·01%

mercuric chloride and germinated on a wet filter paper for 16 h in dark. Fifteen uniformly germinated seeds were transferred to petri dishes (9 cm dia) containing 10 ml standard mineral medium (SMM) prepared according to Doddema *et al* (1978) containing 50 mg l^{-1} GA_3. For control, seedlings were grown in SMM alone. After 1 h incubation in dark the petri dishes were transferred to continuous light (700 Lux maintained by fluorescence tube light). The culture medium was changed after every 24 h. After 72 h, seedlings were rinsed with deionised water and transferred to fresh SMM containing different concentrations of KNO_3 and incubated in it for 6 h.

Nitrate was measured spectroscopically according to the method of Doddema *et al* (1978) by recording UV absorption in Beckman Spectrophotometer Model 26 of uptake medium at 0 h and 1 h after NO_3^- uptake by seedlings. Depletion of NO_3^- in uptake medium was taken as a measure of NO_3^- uptake. The method was found sufficiently sensitive to measure concentrations in the range of 10^{-6} to $0{\cdot}2 \times 10^{-3}$ M NO_3. The most appropriate wavelength was found to be 210 nm and NO_3^- concentration below 0·2 mM can be measured with sufficient sensitivity at this wavelength. All the concentrations were brought to 0·2 mM or less after suitable dilution. The ions present in SMM did not interfere except undissolved $CaCO_3$ which gave measurable absorption when SMM was measured against distilled water. However, base line could be adjusted to zero when absorption of SMM was measured against SMM. Therefore, all the concentrations were measured against SMM control. Since some organic compounds also absorb at the wavelength used in this experiment and thus can interfere with the determination. Attention has been paid to the possible leakage of material from the seedlings, however, such leakage was found to be negligible for short time experiments (uptake period 1 to 2 h).

Actual *in vivo* NRA was measured as *in vivo* NRA except that the incubation medium did not contain NO_3^- as described by Breteler *et al* (1979).

For NO_3^- determination, seedlings were frozen immediately and ground with mortar and pestle in 4 vol of 0·1 M K-phosphate buffer (pH 7·4) and centrifuged at 25000 *g* for 15 min. NO_3^- in the supernatant was assayed by hydrazine reduction method (Fishman *et al* 1964). Reduction of NO_3^- was determined by subtracting the total amount of NO_3^- accumulated in the tissue from the total taken up during the uptake period.

Soluble sugars were extracted by grinding seedlings with 80% (v/v) ethanol. The extract was filtered through Whatman filter paper No. 1. The filtrate was heated to evaporate the ethanol. After acid hydrolysis (Smith 1969), total sugars were estimated with Millers reagent (Miller 1959).

Hill-reaction activity was measured by the method of Vishniac (1975) and total chlorophyll was estimated according to Arnon (1949). All the experiments were performed in triplicate and the mean values are presented.

3. Results and discussion

Uptake and reduction of NO_3^- showed saturation kinetics when plotted against ambient NO_3^- concentration. In low NO_3^- concentration (below 2 mM), NO_3^- uptake and reduction showed concentration-dependent increase, however, in higher concentration, uptake as well as reduction showed rate saturation (figure 1A). The concentration effect of NO_3^- on both uptake and reduction is the same since the rate of NO_3^- reduction is reported to be a simple function of the rate at which

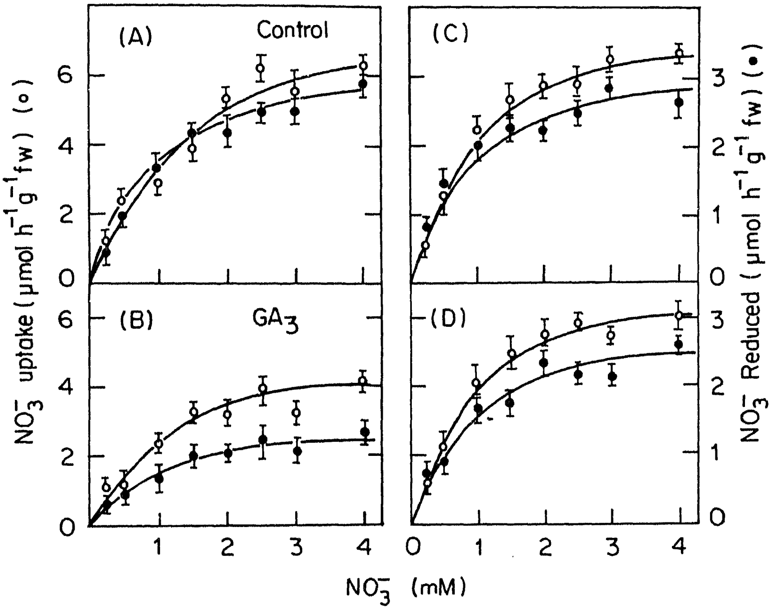

Figure 1. **A, B.** NO_3^- uptake and reduction by control and GA_3 treated 72 h old *Sesamum* seedlings. Induction medium contained different concentrations of NO_3^- without sucrose. **C, D.** 2% sucrose (w/v) was added in the induction medium along with different NO_3^- concentrations.

inducer becomes available (Breteler *et al* 1979) and both uptake and reduction of NO_3^- are induced by the same substrate, viz. NO_3^- (Jackson 1978).

GA_3 treated seedlings also showed concentration dependent saturation kinetics, but uptake and NO_3^- reduction were low as compared to control seedlings. However, in both processes rate saturation occurred almost at the same substrate concentration as in control seedlings. NO_3^- uptake was nearly 33% less while its reduction was 52% less than control seedling at 2·5 mM ambient NO_3^- concentration.

Reduction of NO_3^- in plants is catalysed by an enzyme NR (EC 1·6·6·1) (Beevers and Hageman 1969). Breteler and Hänisch ten Cate (1980) observed that actual *in vivo* NRA is a good measure of *in situ* NO_3^- reduction. In the present work also actual *in vivo* NRA was in good agreement with *in situ* NO_3^- reduction both in control and GA_3 treated seedlings (table 1). In plants NO_3^- uptake and NRA were found to be causally related by several workers (Rao and Rains 1976; Jones *et al* 1981).

Various Kreb's cycle intermediates and sugars are known to enhance the reduction of NO_3^- in dark in sugar deficient tissue (Aslam and Huffaker 1984). Reports from other sources also indicate that the induction of NR is energy requiring process which is either supplied by light through photosynthesis or from photosynthetic products like glucose, fructose, sucrose etc (Aslam *et al* 1976, 1979). Therefore, it is necessary to interrelate tissue sugars with NO_3^- reduction. Total sugars were recorded much lower in GA_3 treated seedlings as compared to control. By appearance GA_3 treated seedlings were pale and showed considerable inhibition

Table 1. Effect of sucrose 2% (w/v) feeding on NRA in control and GA_3 treated *Sesamum* seedlings. The results are expressed as a per cent of control (−sucrose) seedlings.

NO_3^- Conc (mM)	Control + sucrose	NRA GA − sucrose	GA + sucrose
0·25	112·5 ± 4·2	51·5 ± 7·6	66·25 ± 7·1
0·5	104·8 ± 3·8	47·6 ± 3·2	67·18 ± 7·3
1·0	105·2 ± 11·6	34·6 ± 4·3	53·11 ± 3·7
1·5	116·6 ± 9·2	45·2 ± 3·7	76·14 ± 11·1
2·0	114·1 ± 5·1	43·2 ± 8·1	78·22 ± 2·7
2·5	95·3 ± 7·2	41·36 ± 2·5	75·00 ± 8·5
3·0	110·1 ± 11·8	45·7 ± 3·4	92·16 ± 9·6
4·0	109·2 ± 13·2	48·6 ± 6·5	88·46 ± 8·5

Table 2. Effect of GA_3 on total sugar, total chlorophyll and Hill-reaction activity in 72 h old *Sesamum* seedlings.

Treatment	Total sugar ($mg\ g^{-1}$ fw)	Total chlorophyll ($mg\ g^{-1}$ fw)	Hill-reaction activity μmol DCPIP reduced ($min^{-1}\ g^{-1}$ fw)
Control	5·58 ± 1·3	0·86 ± 0·08	2·75 ± 0·43
GA_3	2·78 ± 0·7	0·51 ± 0·06	1·36 ± 0·27

in Hill-reaction activity and total chlorophyll content (table 2). This might indicate poor photochemical activity and therefore, account for low sugar content in GA_3 treated seedlings. To confirm the regulatory role of sugars in GA_3 treated seedlings, 2% sucrose (w/v) was added in the incubation medium. The results indicate that both uptake and reduction of NO_3^- showed marked reversal of GA_3 induced inhibition and brought it almost to the level of control seedlings (figure 1D). However, there was no significant promotion of NO_3^- uptake and reduction by sucrose feeding in control seedlings (figure 1C). Therefore, sucrose promoted NO_3^- uptake and reduction in sugar deficient tissue, which in present case are GA_3 treated seedlings. Control seedlings appear to have optimum level of sugar contents. Aslam *et al* (1979) reported inhibition of NO_3^- uptake and reduction in the absence of light in carbohydrate deficient tissue. However, the inhibition in NO_3^- reduction was more than the inhibition in NO_3^- uptake. Higher inhibition (52%) of NO_3^- reduction as compared to NO_3^- uptake (33%) was also found in the present study. NO_3^- uptake is involved in dynamic interaction with its reduction and accumulation. It has been found inversely correlated with tissue NO_3^- concentration (Breteler and Nissen 1982) and further NRA can influence NO_3^- uptake rate by changing tissue NO_3^- concentration (Lüttge and Higinbotham 1979). Similarly NO_3^- accumulation and reduction in tissues are mutually dependent (Aslam *et al* 1987). Therefore NO_3^- accumulation affect both its uptake as well as its reduction. Average NO_3^- accumulation/uptake ratio was higher for GA_3 treated seedlings as compared to control seedlings (table 3) indicating that accumulation per mol NO_3^- was nearly 29% more in GA_3 seedlings.

From the present study it can be concluded that GA_3 inhibited NO_3^- utilization

Table 3. NO$_3^-$ accumulation/uptake ratio in control and GA$_3$ treated *Sesamum* seedlings.

NO$_3^-$ Conc (mM)	NO$_3^-$ accumulation/uptake	
	Control	GA$_3$
0·25	0·49 ± 0·03	0·70 ± 0·05
0·5	0·45 ± 0·14	0·66 ± 0·08
1·0	0·44 ± 0·04	0·67 ± 0·06
1·5	0·55 ± 0·07	0·73 ± 0·09
2·0	0·59 ± 0·08	0·68 ± 0·13
2·5	0·54 ± 0·07	0·77 ± 0·05
3·0	0·54 ± 0·12	0·69 ± 0·02
4·0	0·51 ± 0·08	0·67 ± 0·07

in *Sesamum* seedlings, which is evident from their poor uptake and NO$_3^-$ reduction rate. Since GA$_3^-$ treated seedlings were found to contain low tissue sugar and ambient sucrose feeding could significantly reverse the GA$_3^-$ caused inhibition of NO$_3^-$ uptake and reduction, it is suggested that this inhibition is caused by low level of sugar of the seedlings which in turn may be the effect of their poor photochemical activity.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 6, December 1988, pp. 425–464.
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Plant tissue culture: Perceptions and realities*

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MS received 22 June 1988

Abstract. There is a perception that phenomenal progress has been made in so-called tissue culture technologies as they apply to non-woody plants. Moreover, many also seem to think that while temperate woody species have yielded to *in vitro* manipulation, tropical and subtropical ones have generally remained recalcitrant. An attempt has been made to place all this in context, and to allow for emergence of a realistic conclusion of where we really seem to be. Much progress has been made but plant tissue culture procedures still have a long way to go before they can collectively qualify, by the most rigid criteria, as a routine tool. If a technology is, in fact, a means to amplify our ability to do something in a relatively, if not virtually, risk-free manner, anyone who has done much serious plant tissue culture will know that in most cases it does not yet meet the criterion. When we can predictably control things—with maximal certainty, risk-free, or at least with minimal or acceptable risk, we will have a biotechnology. It sounds very modern to use the word 'biotechnology' but one does not achieve the status of a biotechnology merely by many or most agreeing to use the term. My belief is that we work against ourselves by doing so. Basic scientific inquiry is stifled. We still need to provide an appropriate framework and vehicle for basic investigations that will enable many important unanswered questions to be addressed. From a practical perspective these include: Why does progress seem to be slow in woody plants—especially in the case of tropical and subtropical species; what are some of the constraints imposed on the systems being worked with; how much of a gap is there between knowledge at the research level and application; how can one accelerate activities to improve both our perspective and still make positive basic and practical contributions to this area? A major problem has been that some systems are quite far along in their development; others are in their infancy. It is hoped that supporters of plant tissue culture efforts will not abandon basic research programs in favor of applied initiatives just because some species seem to be more difficult to work with initially than others and hence will require much more time at the basic research level, and well before one can begin to develop and ultimately exploit. Basic botanical and plant cell biology research must be fostered. Those dedicated to studying plant biology from an *in vitro* perspective have a greater role now than ever to play. Responsible action is crucial. Resistance must be given to those who prematurely insist that integration into practical schemes should predominate our thinking and setting of priorities. At the same time there is a delicate path that must be taken so that opportunities for learning and making basic contributions are not missed. Plant scientists clearly have a challenge to meet. Decision making must take into account the state of the science from the scientists' perspective—not merely an administrative perspective.

Keywords. *In vitro*; tissue culture; tropical plant biology; cloning; somatic generative approaches; biotechnology.

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*This paper represents an edited and revised transcript of a profusely illustrated plenary lecture presented in Kuala Lumpur, Malaysia at a Seminar on Tissue Culture of Forest Species. Attempts have been made to preserve the flow of the talk despite the use here of limited illustrative material. Publication of this updated version is made possible through the kind invitation of the Chairman of the Editorial Board, Proceedings (Plant Sciences) and Prof. A N Rao, National University of Singapore, Singapore.

1. Introduction

So much is being written about the use of aseptic culture techniques and biotechnology for plant improvement and management schemes that it is virtually taken as given that methods are routine and applicable as tools for a sufficient number of species to make it a worthwhile and high priority activity. Some sincerely believe that financially remunerative enterprises that are based on the new plant biotechnologies exist in large numbers throughout the world. In a word, plant tissue culture and related 'high tech' activities have become commercial.

The fact is that there are very few plant biotechnology operations anywhere producing wealth. Profits from 'sales' have been far less than from stock sales and speculation, contract research and joint ventures. This will remain so in my opinion for some years to come. In a word, activities performed in establishments incorporated to do business are different from profit-generating businesses based on sales of product or services.

One sees at the outset of this paper, therefore, that my intent is to put forward some rather controversial viewpoints. Undoubtedly there is still a huge disparity among many of us as to what each of us thinks plant biotechnology 'really is'. This is not a simple problem to surmount. I will try to address some of the issues as they relate to one small area of 'biotechnology'—namely that of plant tissue culture. The plant molecular biology and genetic aspects will not be covered here. But since in many cases the molecular biology is intimately tied to the tissue culture or cell biology, the points I am trying to make will hopefully be made adequately clear if we focus on the plant tissue culture problems.

There is such a well-developed and established infrastructure, if you will, of publicity generated by the lay and science press that it takes courage to go against conventional wisdom and to try to separate authoritatively fact from fantasy, and reasonable possibility from actual achievement. It is especially difficult in the milieu of the past several years even for so-called experts to assess what has really been going on. For many, all of it is 'new'. Some may, in fact, be surprised to hear that the word biotechnology was in use as early as 1920 (Dixon 1985). Plant tissue culture procedures date from that period as well (Krikorian 1982; Torrey 1985).

For me, it has come down to a theme of something like 'perception versus reality'. If a majority perceives something to be so, then it seems to achieve the status of reality. This would seem to be a sort of tacit agreement to do 'science by committee'—virtually forging ahead by other than scientific means—[legislating if you will, scientific 'progress']. Far too few scientists have spoken out against the abuses. The proponents of biotechnology and its followers have so effectively and so thoroughly carried out their communicative activities, that for quite a few years, many who were not directly involved in such work were led to believe that virtually all the major problems of plant tissue culture had been solved and had been reduced, as it were, to the level of a 'technology'—whatever that meant.

Part of the perception was naively generated by over-enthusiastic scientists attempting to simplify their research activities for non-specialist audiences, and part of it by ill-informed, albeit well-meaning, bureaucrats seeking to justify their roles as nominal advocates of the science. Some administrators insist that any means whereby funds can be made available for research is justified. We are told that everything turns on programmatic initiatives. Federal agencies are constantly under pressure from the money-givers (in the US, Congress and their constituents) to show rapid gains from money expended. (One of a number of arguments that I can make against this stance is that areas still in need of support are usually pushed aside to make way for the 'more important' initiatives!). A particularly sensitive and arguable issue is that an all-too-large and effective role seems to have also been orchestrated by clever 'businessmen' to generate profits. This was particularly so a number of years ago when much of this whole business was taking form. The views of more conservative and experienced plant tissue culture workers such as myself were, in retrospect and perhaps predictably, too frankly and disturbingly objective (Krikorian and Steward 1969; Steward and Krikorian 1975, 1979; Krikorian 1982) to have had much success in quelling the tide of propaganda heralding nominal successes, both scientific and commercial, on a near-daily basis.

Time has, in my view, forced a more considered evaluation of both the potentials and real accomplishments. Most responsible scientists have 'come down to earth' and the weaker commercial enterprises who have not had the protective cushion of a parent organization—frequently on the order of a 'superstructure'—have left the scene and expectations are no longer so much out of scope with the economics. No major and quick profits will be made—at least based on sale of real products or delivery of services for which informed cutomers will pay (e.g. Day 1986; Crawford 1986). Even the better established companies will have pressures to meet production costs (Jones 1986).

2. Context

Figure 1 from Simmonds (1983a) gives an overview of the relationship of some of the activities that one might project between *in vitro* methods and plant breeding. On the left hand side, *in vitro* techniques are presented, and on the right hand side, separated by a dashed line, we see genetic engineering potential. We start off, at the top of the scheme, with a crop plant. For our purposes, we can use as an example a tree species, or woody plant, it really doesn't make much difference. We then move to the boxes and circles which relate to tissue culture in the generic sense of the term—that is everything ranging from, say, protoplast culture all the way to the aseptic cultivation of an excised organ or even an intact plant. We can use excised orderly meristems—that is to say, pre-formed meristems, or shoot tip cultures. We can also carry out embryo culture. Embryo culture, of course, has been used for many years to a greater or lesser extent for the rescue of progeny of difficult crosses (Williams *et al* 1987). The late Professor Harold B Turkey Sr. was the person in the western hemisphere who popularized the embryo culture technique for woody species—specifically stone fruit crops (Tukey 1944; Narayanaswamy and Norstog 1964). Shoot tip culture has, as we will see in a moment, been used in the production and multiplication of disease-free material. People are also beginning to look into the value of the methodology for genetic resource collection, conservation and preservation (Kartha 1985; Schaefer-Menuhr 1985). If we look at the upper right of figure 1, where unorganized calluses, cells, protoplasts and what I call organized propagules are listed, we see this has a connecting bridge to the other *in vitro* techniques and the genetic engineering potential in that we think we can use these methods. However, there is a question mark and a block at this crucial point.

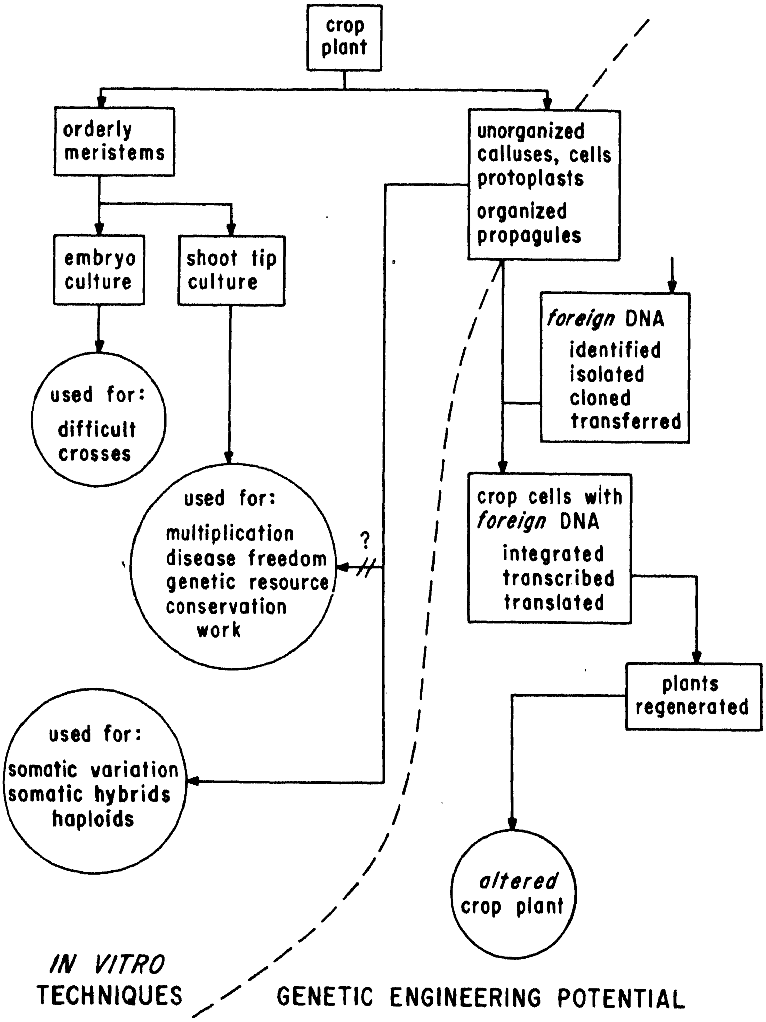

Figure 1. Plant breeding and the use of *in vitro* methods. Redrawn with permission from Simmonds (1983). See text for details.

But we have a connecting line from 'unorganized callus, cells, protoplasts and organized propagules' shown at the upper right down to the lower left hand corner where their use is implicated for 'somatic variation' (or what I prefer to call culture associated variation), production of 'somatic hybrids, haploids etc'.

We have all seen these kinds of schemes put forward in different ways, and presented in different places. Simmonds (1983a, b) developed this scheme for use in a very conservative, and accurate in my view, assessment of the usefulness of plant tissue culture for short term practical gain and genetic engineering (see later). More than several years after development of the scheme we may still ask to what extent is all this 'reality' and to what extent is it 'perception'. I think it behooves all investigators to put in perspective for themselves where and at what level they think they are working. In that way one will become better able to communicate with one another and thereby perhaps make some progress. If one doesn't recognize or admit where there are real problems and obstacles, one cannot make the first step towards solving these.

I fault the tissue culturists in no small measure for being perhaps a bit too enthusiastic in heralding the achievements and accomplishments without drawing appropriate attention to the still basic research aspect of our work. We would like very much to talk about routine 'development' and 'applications' and 'grassroots use' but in most cases we aren't yet ready. I think there is a lot of proof for this view. The minute we try to jump ahead and to legislate, as stated above, scientific progress, we end up in difficulties. I think it is salutary at this point in time that we are undergoing a readjustment and we need frequent and open communication in looking seriously, on a continuing basis, at where we really are. I don't think that we can do this effectively unless we openly talk about the kinds of things that one might even be trying to achieve when viewed from a practical perspective.

This leads me to a second objective of this overview. That is, we want to be respectful, as it were, to what has been done from a scientific and technological perspective, but we do not want to over do it and claim successes that are not warranted. Succinctly put, given the descriptive, often empirical knowledge currently available, our ability to identify and apply general approaches for production is a universal frustration for anyone seeking to work with all but the 'easiest' tissue culture 'systems'.

If so-called biotechnology as it relates to things we in plant tissue culture are often interested in were a reality, we would all have at hand, controllable, technical methods of achieving a given practical purpose. The biotechnology would amplify our ability to do something in a relatively, if not completely, risk-free manner. We would be able to embark upon highly predictable so-called 'process technology' programmes. We would know what we wanted, more or less, how long it would take to achieve it and would be able to effect change in detailed or precise but important ways certain specifications in a product. We would be pre-occupied with improving performance, cost or to make things more streamlined. Instead, in my view, we are embarked upon programmes which are highly uncertain, albeit very exciting and interesting. We do not always know where we are going when we start. We often don't know how to go, or get there, and we don't necessarily know what the final end-product will be. In short, we are generally involved in basic research (Cocking 1987).

Plant tissue culturists will recognize against this superficial background that we ire still exploring and carrying on basic research seeking both fundamental and echnological breakthroughs. At the same time, new industries are struggling to be reated and to survive. 'New products to new markets' is a recurrent theme familiar o most even though it may not have been verbalized as such. Plant tissue culturists vill also either know, or intuitively suspect, that there is a broad spectrum of ctivity in progress worldwide in diverse quarters which is not necessarily equal in mount, intensity or even importance. In a word, plant tissue culture is striving to e partly a process or technique technology, and partly a product technology. As pecialists, we need to couple the two and to lengthen the continuum. If 'innovation' s applied development or exploitation of 'invention', then we as basic scientists are bligated to contribute to the invention process or at least to try to understand the uances of the innovation process. We need to study the relationships to the redicting and optimizing of applications.

All will recognize that there is complexity in all aspects of human interactions. Humans will be humans. At one extreme we have the inflated 'tissue culture mystique'. Here we are criticized as practicing some sort of 'black magic or art', and hence are accused by some of being unscientific. At the other extreme we have those who state, categorically, that we already represent a bona fide biotechnology ripe for immediate and risk-free exploitation. Some are even raising ethical and social issues that exist or will shortly arise due to existing realities (e.g. Hansen *et al* 1986; Panem 1986). We can also cite examples of political steps being taken to control biotechnology, and attempts even to use it as a lure or weapon of sorts. Controversies over plant germplasm have arisen and provide a glimpse of the issues that will have to be resolved (Kloppenburg and Kleinman 1987).

Inevitably, the perceptions are for the most part wrong. The reality lies somewhere in between the extremes.

3. Prospects

A few years ago, I tried to look into a crystal ball and project a list—albeit without a specified timeline—of activities that currently available tissue culture techniques could facilitate. (Note my careful use of the word facilitate. It implies that one does not seek to replace; rather, one seeks to amplify and give another dimension to, existing approaches). When one takes the attitude that tissue culture is going to replace or supercede existing methodologies, we do a disservice to all concerned. There is a sobering effect that experience has forced on all those who would have espoused the view that tissue culture would take the lead in the 'second green revolution'. Nevertheless, I think it is perfectly reasonable to state that we are able with greater or lesser ease to accomplish certain tasks (table 1).

4. Rapid multiplication of select specimens

We can achieve rapid multiplication of select specimens in certain instances; in other cases we can eliminate viruses—if we know what the virus is to begin with; we can even eliminate viruses that we don't know the identify of using double-stranded RNA techniques (Jordan 1986). We can index viruses if we are careful and patient enough. It takes a much longer period of time to do virus indexing than most people are willing to acknowledge. In the pelargonium (geranium) industry of the United States it may take several years to produce an indexed line of plants in quantities sufficient for the market (Oglevee-Donovan 1986). All this takes effort and commitment. This is another facet that we must be very careful about. What time frame are we talking about? Is the answer to the question 'yesterday'—which is undoubtedly what we would all prefer to see or hear? Or, is it 'downstream'—only realizable within several or even many years? For bunchy top virus of bananas, a major problem in India, the idea is that it will take some time and a fair amount of effort to have adequate procedures in hand (Dale 1987).

5. Germplasm introduction and evaluation

We often say it is much easier to accomplish this with tissue cultures but this is in theory only. It will have to become a reality. I have been engaged in some activities

Table 1. Range of activities that currently available tissue culture techniques can facilitate.

- Research with potential for near term impact culture techniques can facilitate:
  - Rapid multiplication of select specimens
  - Elimination of virus and specific pathogens
  - Virus indexing
  - Germplasm introduction and evaluation
  - Germplasm collection, preservation and management
  - Production of polyploids, haploids, somaclonal variants for new crop production and use in breeding, etc.
  - Elimination of certain breeding barriers
  - *in vitro* fertilization *in ovulo*
  - embryo rescue and/or storage
  - androgenesis
  - gynogenesis
- Research with intermediate impact
  - All the above in more recalcitrant species, plus
  - selection for complex traits such as tolerance to stress
  - biotic—diseases and pests
  - abiotic—temperature
    - salt
    - herbicides
  - *In vitro* mutation breeding
  - Cryopreservation
- Research with long range implications
  - All the above in still more recalcitrant species, plus
  - genetic engineering
  - transformation by selectable genes etc.
  - organelle transfer
  - wide crosses-somatic hybridization
  - Understanding controls in developmental and physiological processes

trying to move germ plasm around and I can guarantee you that it is not as easy as one would hope or imagine. In fact, I presently do not know anyone who has done it who says it is simple. It is easier to the extent that a tube(s) can be moved easier than a heavy crate (Hewitt and Chiarappa 1977; Kahn 1986). But the machinery is not very often in place to allow one to accomplish this easily. Even now, years after the whole got off the ground, one frequently encounters the embarassing situation of scientists and other formally authorized individuals tucking and secreting culture tubes in their pockets or purses to effect germ plasm transfer. Quarantine officers and agricultural inspectors at various ports of entry or at airports all too frequently have no or insufficient ideas as to how cultures should be examined or assessed. Many want to open aseptic culture vessels. Undoubtedly, more than 'fine tuning' of the legal aspects and education of agricultural quarantine inspection officers needs to occur (Parliman and White 1985).

Moreover, one cannot assume, just because something is *in vitro* or *in plastico* that it is free of contaminants or infestation. Certainly there is no guarantee that a culture is specific-pathogen free. All too many 'tissue' cultures that I have seen in circulation have not even been rigorously assessed in terms of being bacteria-free or fungal-free (Schaffner 1979). The unwarranted assumption seems to be that a culture is clean because it hasn't become visibly contaminated. Only if a bacterial and fungal culture medium is used to check, repeatedly, the cleanliness of a given culture (and this includes cutting of materials to expose inner surfaces to an appropriate microbiological nutrient medium and incubation at elevated temperatures permissive to the growth of these infestations) can one be satisfied that a culture is acceptably bacteria or fungi-free. Simply put, material is not clean just because it is *in vitro*. I have been shocked to see how few people rigorously assess the status of their cultures (Knox and Smith 1980). It may not matter if a culture is in an organized growth mode—say a shoot tip or meristem if a slow growing or cryptic contaminant is present (Young *et al* 1984). But if one attempts to work with callus, cells and protoplasts, even cryptic contaminants can preclude success. All this emphasizes that one should not take anything on face value. Although we are scientists, we sometimes want to assume things. There is nothing that should be assumed. There is no basis for assumption. You prove something. You demonstrate that something is free of specific bacteria or fungus etc. The view that cultures can never be truly axenic ought not to deter us from eliminating those agents that we can.

6. Production of polyploids, haploids, somaclonal variants (or tissue culture-associated variants) for new crop production and use in breeding etc

There should be no-one, anywhere, who assumes that everything or even much of what is produced from *in vitro* manipulations is going to be used directly as a product in forestry or agriculture. Tissue culture-generated plants should be seen more as 'ports of entry' into the conventional process of breeding and selection. There are relatively few things—one can think primarily of floricultural or ornamental crops—that will directly become products. Cultures are more properly viewed as the starting point for further genetic manipulation via more conventional plant breeding activities. Tissue culture-derived products will more normally feed into the beginning of the agricultural-horticultural-forestry pipeline; they will generally not be the end of the pipeline. In my view one will, for the short term and from the agricultural perspective at best, feed new things to the breeders (Evans 1986). Woody plants and forest species will also predominantly follow this pattern but there will be exceptions, of course. A conspicuous consequence of all this is that it serves to lengthen further the time frame within which tissue culture could possibly have a truly significant impact.

One can legitimately ask 'why don't we just carry out conventional breeding?' This is a good question. The fact is that there are too few adequately trained breeders—especially in less economically developed countries. In the developed countries, some 'classical' breeders are taking early retirement because they are frustrated by the position often taken by their administrators that they should 're-tool' and 'modernize'—they should use more 'biotechnology'. This situation is untenable and is sure to have serious detrimental impact in the near future. Childers (1986) has already pointed out that there is a developing crisis in traditional horticulture. Surely we do not want to have a field flooded with 'biotechnologists' who have inadequate scientific 'corporate memory' and appreciation as to where the real problems lie. It would be analogous to having to 're-invent the wheel'. The availability of an underexploited genetic reservoir of edible-fruit tree species in various centers of origin has been emphasized and re-emphasized (Terra 1952; Jong *et al* 1973; Bose 1985; US Congress, Office of Technology Assessment 1987). Exploitation of this would seem to be a worthwhile activity. Too many people attempt to justify tissue culture for the sake of tissue culture and all too frequently one asks, 'why are you tissue culturing such and such a plant?', and, in turn, only a blank stare is elicited. Surely a particular tissue culture activity should be thought through more critically!

7. Elimination of certain breeding barriers

In vitro fertilization *in ovulo*.
Embryo rescue and/or storage.
Androgenesis.
Gynogenesis.

Much has been written about these but, again, we really do not know very much (Chapman *et al* 1986; Han and Yang 1986; Johri 1982; Raghavan 1986). All of these approaches in time will perhaps have their select place. But I often wonder whether the real advances will derive from such approaches and applications along the lines predicted or enumerated in table 1. Obviously, I really do not know if the listings will have very much to do with 'reality' from a substantive viewpoint, or whether they are and will remain 'perceptions'. Once again we come back to this recurring theme of perception versus reality. The approaches listed in table 1 sound good. Now the problem is to select a system and to see if we can make it work. Is the approach applicable or practicable under a particular set of circumstances? Clearly, the activities cannot be applicable 'across the board'. You cannot be 'all things to all people all of the time'. And I think this is again one of our mistakes. We fail to qualify adequately our efforts and to appreciate the limitations at hand.

Now if we talk about 'intermediate impact', we can state 'all of the above in more recalcitrant species or materials'. What do we mean by recalcitrance? We all know that there are things which are easy and those that are difficult-to-work-with. We shall talk about whether that is real or not a bit further on.

— Selection for complex traits such as tolerance to stress.
Table 2 shows that activities related to stress research are complicated in their own right. It is certainly not a casual affair. We talk about biotic versus abiotic.
— *In vitro* mutation breeding.
— Cryopreservation.

We are nowhere near, in my view, achieving cryopreservation with the level of confidence that we would like (and require). We cannot preserve things for practical purposes unless we know that which we recover from the preserved state is what we want. This takes time to develop and assess (Kartha 1985). It is bad enough for temperate species (Stushnoff and Fear 1985), and tropical ones will be more difficult to deal with.

8. Research with long range implications

'— All the above in still more recalcitrant materials'.

Table 2. Objectives of efforts to select and/or develop stress-tolerant plants.

- Develop protocols for identifying stress or environmental factors that alter function and productivity
- Document the effects of various stress regimes or situations on physiological, biochemical and biophysical processes
- Develop genetic screening procedures in t ıne with the above processes so as to identify the existing range of variability
- Develop cooperative research with breeders and geneticists to ascertain the basis and mode of inheritance of any stress tolerance
- Utilize this basic information to select and develop germplasm and growing methods that will avert or minimize negative impact of stress

Plus what has come to be called 'genetic engineering', whatever that may really mean (Board on Agriculture, National Research Council 1984). 'Transformation by selectable genes etc'. There are, arguably, very few single gene traits worth selecting for (Goodman *et al* 1987). They can be used as tools for research. There are not too many that have been identified where a single gene is going to make a major difference (Shah *et al* 1986). In fact, one is not always sure what one really wants in a given plant. We are still learning (Davies 1977). There is a great deal of germ plasm already available to breeders that has not been adequately exploited. And if this 'gene pool' were utilized fully we could be very well-off indeed (Simmonds 1962; Wilkes 1983; Plucknett *et al* 1986). Also there are many existing ecotypes that could be tested and evaluated at various locations without ever having to be 'engineered' Hackett and Carolane 1983 and updatings; Hackett 1987).

'— Wide crosses—somatic hybridization'.

This has been the subject of a great deal of discussion and controversy. An article published several years ago in the *New Scientist* entitled 'The revolution that failed' draws attention to the lack of progress in an area that nominally held great promise (Burgess 1984). All sorts of wonderful things had been projected by otherwise responsible and well-known scientists from activities involving somatic hybridization of protoplasts (e.g. Cocking *et al* 1981). In some cases, protoplast fusion might well be a useful research approach. In some of our daylily (*Hemerocallis*) work, fusion of diploid protoplasts has resulted in tetraploids. Since tetraploids are larger, and have more showey blooms, this is generally seen as a pòsitive development. However positive this may be (Fitter and Krikorian 1988), this select case involving an ornamental plant merely serves as a reminder of the limited application at this point in time on a practical level. In a predominantly clonal plant, derived from many low-fertility hybrids, the approach seems justifiable. Again, and for research purposes, a similar approach with clones of genus *Musa* (bananas, plantains, etc.) can be of substantial interest (Krikorian and Cronauer 1984; Krikorian 1987). The predominantly basic research approach is emphasized by Gleba and Sytnik (1984) and Daub (1986).

'Understanding controls in development and physiological processes'.

This last entry to table 1 is, I feel, the most important. It will more than likely be at this point where the new knowledge that can permit applied work to go forward will emerge. The understanding of the controls in developmental and physiological processes will open the door, as it were, for the kind of achievements that we, who have any kind of practical bent, can grow upon.

Against the background posed above, we are, as investigators interested in plant tissue culture, still seeking fundamental and technological breakthroughs. So we are a bit between what is termed 'the devil and the deep blue sea' because on one hand we are achieving quite a lot and, at the other extreme, we seem to be spinning our wheels. And again, this causes confusion because we have the conflict of perception and the reality. Tissue culturists represent a very broad spectrum of activities not only because of their interests and aspirations, but because of their achievements in different areas of activity. The species we work with also makes a big difference as to where we see ourselves. Surely plant 'biotechnology' deserves to be more than species-specific.

9. Constraints to progress

Clearly there are constraints to progress. In table 3 some of these are listed. Meetings are now being held which bear titles that allude to some of these. One talks of strategies to remove 'bottlenecks to biotechnological progress'. Certainly, the tone of some of these meetings is changing from those held only a few years earlier. Indeed, the inevitable recognition by 'new comers' that there are indeed major bottlenecks may be seen as a major step forward in adjustment of attitudes. 'Constraint removal', 'capacity strengthening' are other terms or 'buzz words' that reflect recognition of the need to transcend existing limitations at the educational level—both theoretical and practical.

It helps no one (especially the very investigators seeking to work with and to understand the many intricacies of plant tissue culture) for anyone to overstate or exaggerate the capabilities of the techniques. Work should be justified because the objectives and broad procedures to be developed or adopted have been carefully thought through. Moreover, it should be said that a specific goal oriented project is, at the outset at least, being carried out as a research effort. It is essential that workers resist with vigor, all pressures to push prematurely what are essentially research projects into the development or production mode (e.g. Cohen *et al* 1988). This is where a major challenge presents itself to researchers (Rachie and Lyman 1981; Collins 1982). Workers themselves must first fully understand what basic research is, and, they must then appreciate its difference from development and production. Prematurely pushing a laboratory project to the production mode, not only does the science a disservice, it discloses a fundamental failure in one's understanding what the biology and methodology entails and what specific and

Table 3. Some limitations and constraints to progress in the use of plant tissue culture.

—Investigators and support personnel with adequate training and scientific background
—Financial resources
—A bureaucracy sympathetic to research with a long 'lead-time' to 'development' and administrators able to facilitate implementation of programs
—Adequate release time in universities from undergraduate teaching duties so that principle investigators can work in the laboratory
—Recognition not only of capabilities but limitations of 'tissue culture' techniques
—Teamwork at individual, institutional, local, national and international levels
—Maximum integration with other disciplines
—Frequent communication is in process but open communication is a limited commodity

discrete stages need to be addressed before practical applications become a reality. Stating that we have to start somewhere is no appropriate argument. We have to start at the basic research level!

Whereas most scientists have traditionally withdrawn from taking active roles in what are essentially science policy—'political,' if you will—decisions, the time has come for investigators to speak up and to participate actively in educating the public and policy makers as to the details of their science—all this even as they do their science (Teich 1974; Westin 1970, 1981; Gibbs and Carlson 1986). 'Those who will not lead, will be led'. Many can recount too many stories about bad decisions being taken because overzealous administrators failed to 'read the science' accurately; simultaneously, the investigators themselves neglected to analyze the facts of the case accurately (Kenney 1986).

Teamwork at individual, institutional, local, national and international levels is critical to success. It is not easy. One has to work at this. If, as has been said, 'man has been put on Earth so as to rise above human nature', then we may assume that with considerable effort we can learn to work with one another.

Maximum integration with other disciplines is a major need. Even now too many people claim that their primary scientific interest or area of concentration is tissue culture. It would be more readily appreciated by most scientists at large, I think, if one described one's interests as cell biological, physiological, horticultural, genetic, biochemical, morphological etc. While we are still all too often just learning to culture many plants, organs, tissues, cells and protoplasts, the ultimate hope is to be able to use reliably and predictably the various culture methods as scientific tools. Tissue culture is, for the most part, a technique still in its early stage of development, not a discipline. When there is maximal integration and co-ordination with more traditionally defined areas, the methods take on more meaning and profit from the broader context. A broad scheme, reproduced from Thompson (1985), into which those who are more practically oriented could integrate is provided in figure 2. The inter-relationships and disciplinary discretions are clearly delineated here and, in this context, tissue culture takes on a very specific significance. Note that in this scheme plant tissue culture methods are seen as playing a role primarily in germplasm enhancement and development. That is not to say that it cannot and will not be of use in other contexts such as germplasm collection and evaluation, cultivar development and commercialization. The real question is when will this be possible for a given plant? Experienced administrators recognize that it will be a generation or two for much of this to be a reality (Brady 1979).

9.1 *Frequent and open communication*

This has, unfortunately, become a problem. Although one can attend and nominally participate in a host of meetings, if people do not communicate, then there is a tendency for one to emerge with the perception that everything has been achieved and that there are no 'problems'. If one 'jealously guards one's ignorance' as stated by Prof. A N Rao of the National University of Singapore, and essentially takes away information from a meeting but does not contribute to it—even perfunctorily—then this too contributes to the generation of erroneous perceptions as to where we really are. The science cannot help but advance if we share information and learn

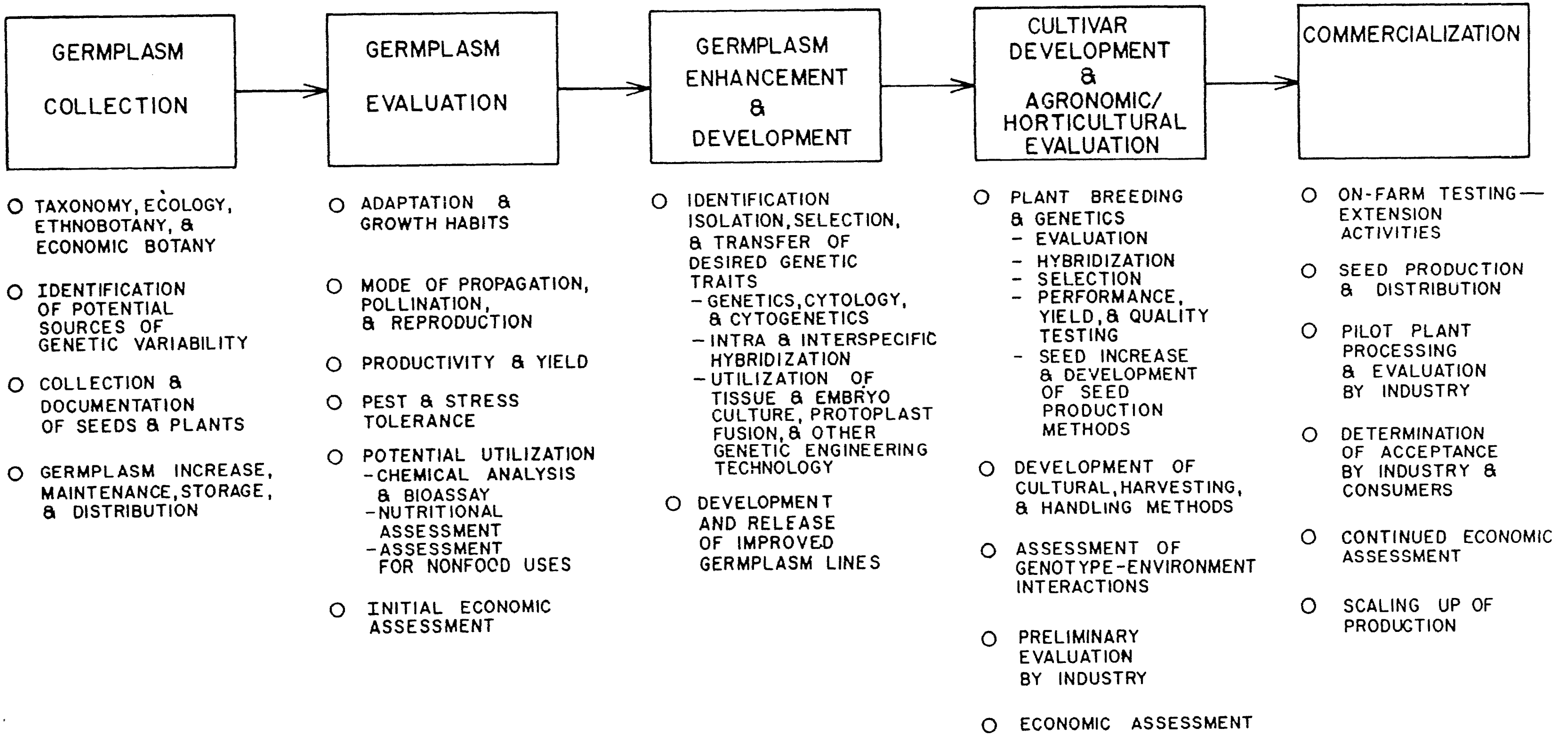
GERMPLASM COLLECTION
GERMPLASM EVALUATION
GERMPLASM ENHANCEMENT & DEVELOPMENT
CULTIVAR DEVELOPMENT & AGRONOMIC/ HORTICULTURAL EVALUATION
COMMERCIALIZATION
O TAXONOMY, ECOLOGY, ETHNOBOTANY, & ECONOMIC BOTANY
O IDENTIFICATION OF POTENTIAL SOURCES OF GENETIC VARIABILITY
O COLLECTION & DOCUMENTATION OF SEEDS & PLANTS
O GERMPLASM INCREASE, MAINTENANCE, STORAGE, & DISTRIBUTION
O ADAPTATION & GROWTH HABITS
O MODE OF PROPAGATION, POLLINATION, & REPRODUCTION
O PRODUCTIVITY & YIELD
O PEST & STRESS TOLERANCE
O POTENTIAL UTILIZATION
-CHEMICAL ANALYSIS & BIOASSAY
-NUTRITIONAL ASSESSMENT
-ASSESSMENT FOR NONFOOD USES
O INITIAL ECONOMIC ASSESSMENT
O IDENTIFICATION ISOLATION, SELECTION, & TRANSFER OF DESIRED GENETIC TRAITS
- GENETICS, CYTOLOGY, & CYTOGENETICS
- INTRA & INTERSPECIFIC HYBRIDIZATION
- UTILIZATION OF TISSUE & EMBRYO CULTURE, PROTOPLAST FUSION, & OTHER GENETIC ENGINEERING TECHNOLOGY
O DEVELOPMENT AND RELEASE OF IMPROVED GERMPLASM LINES
O PLANT BREEDING & GENETICS
- EVALUATION
- HYBRIDIZATION
- SELECTION
- PERFORMANCE, YIELD, & QUALITY TESTING
- SEED INCREASE & DEVELOPMENT OF SEED PRODUCTION METHODS
O DEVELOPMENT OF CULTURAL, HARVESTING, & HANDLING METHODS
O ASSESSMENT OF GENOTYPE-ENVIRONMENT INTERACTIONS
O PRELIMINARY EVALUATION BY INDUSTRY
O ECONOMIC ASSESSMENT
O ON-FARM TESTING— EXTENSION ACTIVITIES
O SEED PRODUCTION & DISTRIBUTION
O PILOT PLANT PROCESSING & EVALUATION BY INDUSTRY
O DETERMINATION OF ACCEPTANCE BY INDUSTRY & CONSUMERS
O CONTINUED ECONOMIC ASSESSMENT
O SCALING UP OF PRODUCTION

Figure 2. A generalized scheme for new crop research and development. While the specific context here is cultivar development, the flow applies equally well to woody species, tropical forest trees and/or plants of interest to agro-forestry efforts. Reproduced with permission from *Econ. Bot.* **39** 440, A E Thompson Copyright 1985. The New York Botanical Garden.

from one another. Opportunities are constantly being created by access to new scientific information. There is never a shortage of ideas. The steroid chemist Carl Djerassi has commented that 'some of the things that you think are trivial may sometimes be exactly the trivial things that someone else needs to jump on very quickly'. I frequently think that journal editors often do a disservice to the very discipline they aim to promote when they unrelentingly urge authors to cut down on 'unnecessary detail' in presenting materials and methods and data. We could save much research time by having access to more detail. We frequently do not know what is necessary and what is unnecessary detail. Similarly, putting the best face on one's research findings, without the inevitable reservations, also can cause the unwary to draw the wrong conclusions. The literature has become overwhelming and there is no sign of letting up. Far too much of the plant tissue culture literature is being prematurely published in my view and, as such, is often an indication of what can happen versus what can controllably be made to happen. Maturity of concept and high degree of reliability, a hallmark of the earlier plant biological literature, is becoming a rare commodity in the literature at large and plant tissue culture is no exception (Maddox 1988).

10. Strategy selection and approaches to aseptic culture

Any decision as to what strategy or line of research should be adopted will, necessarily, depend on the objectives. All basic research has several phases. First, the problem is conceived, and its range and importance defined. Secondly, the best experimental materials are found and the necessary experimental methods elaborated. Thirdly, comes the often arduous—even tedious—work of developing the original idea. As knowledge grows, it may be necessary to use the skills of several workers who make a coordinated team. Finally, comes the exciting moment when the work 'pays off' with new knowledge. This is then studied carefully for its practical and theoretical implications. From the perspective of applying all this and of using it as a technology, one cannot but be impressed how much of an effort it must be. Also, 'there is technology and there is technology'! Figure 3 like many other cartoons, reflects the accuracy of the situation. 'Not high tech, not low tech, but the right tech' says it all. While it may seem to some to be 'modern' and on the forefront, or on the 'cutting edge' of scientific activity, to be doing 'high tech' work, the fact is that the procedures that most use are not, in fact, modern inventions (Krikorian 1982; Torrey 1985). Tissue culture procedures cannot, understandably, be based on methods which do violence to fundamental biological facts. They are inextricably tied to botanical realities. Indeed, only by appreciating the biological facts—having what Nobel laureate Barbara McClintock has called 'a feeling for the organism' (Keller 1983)—can one effectively adopt an approach which has high potential for success.

Because there is a tendency for many to speak of tissue culture techniques in a generic way there is confusion, even among practitioners, as to exactly which given strategy has been adopted. Table 4 lists the bulk, if not all, of the strategies currently in use for multiplication of plants via *in vitro* methods.

When one looks at select examples of the non-woody *in vitro* systems, one cannot help but be impressed—somatic embryos of species such as carrot can be generated by the millions; daylilies (*Hemerocallis*) from explanted ovaries or vegetative apices

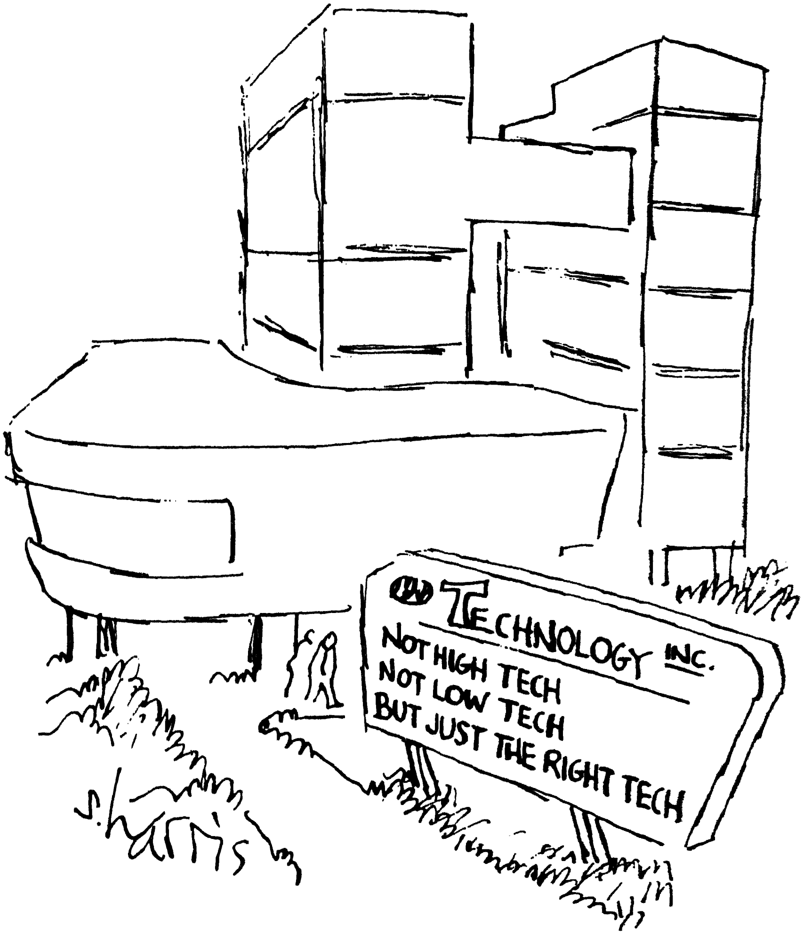

Figure 3. Uncanny insight and wisdom reflected in a contemporary cartoon. Copyright 1987 by Sidney Harris, *Am. Sci.* 75 150. With permission.

also in huge numbers; *Gerbera* plantlets from explanted capitula in equally large numbers; bananas and plantains by the millions; potato plantlets—including fully functional but miniature tubers generated *in vitro*—in huge numbers, are but a few examples one could cite. Since there now is a massive literature that seeks to provide the interface between innovative breeding and various plant management practices, and the challenges and opportunities posed by a particular species, one can do little more than draw attention to the fact that plant tissue culture is here to stay (Ammirato *et al* 1984; Bajaj 1985, 1986; Bhojwani and Razdan 1983; Bonga and Durzan 1987a, b, c; Conger 1981; Constabel and Vasil 1987; Dodds 1983; Evans *et al* 1983, 1986; George and Sherrington 1984; Green *et al* 1987; Pierik 1987; Rao 1982; Sharp *et al* 1984; Vasil 1984, 1985, 1986; Withers and Alderson 1986; Yeoman 1986; Zimmerman 1980; Zimmerman *et al* 1986).

11. Are woody species more difficult to work with than non-woody ones?

Despite the fact that woody species were among those that were early attempted by

Table 4. Strategies for multiplication of higher plants *in vitro*.

- —Shoots from terminal, axillary or lateral buds
  - • shoot apical meristems (no leaf primordia present)
  - • shoot tips (leaf primordia or young leaves present)
  - • buds
  - • nodes
  - • shoot buds on roots
- —Direct organogenesis
  - • adventitious shoot and/or root formation on an organ or tissue explant without an intervening callus
- —Indirect organogenesis
  - • adventitious shoot and/or root formation on a callus
- —Somatic embryogenesis
  - • direct formation on a primary explant
  - • indirect formation from cells grown in suspension or semi-solid media
- —Direct plantlet formation via an organ of perennation formed *in vitro*
- —Micrografting
- —Ovule culture
- —Embryo rescue
- —Mega- and microspore culture
- —Infection with a crown gall plasmid genetically altered to give teratoma-like tumors
- —Infection with *Agrobacterium rhizogenes* to induce roots on traditional cuttings

From Krikorian *et al* (1986).

the pioneers of what might be called the modern period of plant tissue culture methodology development, there has been a long standing perception that woody species are not as easy to deal with as herbaceous ones (Jacquiot 1964; Abbott 1978 and references there cited). It is legitimate therefore, to ask whether there are woody species that are easy to work with? At the same time however, one has to qualify the question and ask whether the perspective is one of micro-propagation from pre-existing developed growing points, or from the perspective of other strategies (table 4). Based on my own experience, there certainly are woody species that are quite tractable for micropropagation. For instance, the *Cinchona* species that yields quinine and related alkaloids seems to be quite amenable to multiplication via procedures that foster precocious branching and subsequent rooting of the propagules (Krikorian *et al* 1982; Hunter 1986). Similarly, poinsettia (*Euphorbia pulcherrima*) has been very responsive *in vitro* to attempts to induce precocious branching. Indeed, our personal experience with poinsettia has been of particular significance since early dealings with latex bearing/yielding species had led me to the view that latex in and of itself was inhibitory to the *in vitro* process (Krikorian and Steward 1969). But I still maintain that success with certain latex-bearing species is insufficient to justify any revised conclusion that the presence of latex is not detrimental to optimal callus growth. An argument can be made that virtually all species in the routine horticultural trade are, of necessity, readily amenable to some mode of multiplication. It also stands to reason that a species which is propagatable by more traditional, non-aseptic culture-based procedures, should be responsive *in vitro*. Our experience is that, indeed, a species which is easy to multiply *ex vitro* will be easy to multiply *in vitro*. I can think of very few exceptions to the statement. In other words, *in vitro* methods may indeed improve, by various degrees, a given response level, and the differences may be quantitatively impressive. But examples where the differences are qualitative are far fewer—that is to say, few really poor responders to conventional methods become impressive responders under aseptic culture conditions.

Such a view, if accurate as I believe it to be, holds special significance for those seeking to multiply a truly wide range of woody species—especially tropical, subtropical and forestry species.

In the case of African oil palm (*Elaeis guineensis*) the responsiveness to *in vitro* manipulation is much more dramatic in the case of embryo-derived or juvenile explants (Nwankwo and Krikorian 1983). When one tries to work with explants from more mature palms, the level of difficulty takes on a new dimension. They are definitely more sluggish in their response (Krikorian and Kann 1986a; Krikorian 1988a). The Chinese tallow tree, *Sapium sebiferum*, (Euphorbiaceae) follows the same pattern. Seedling responses in my laboratory have been rather easy to elicit and precociously branching systems can be sustained. When one undertakes manipulation of explants from mature trees with demonstrated qualities and characteristics (Scheld *et al* 1983), the problem becomes very different. In the first instance it becomes very difficult, virtually impossible, even to get clean explants from field collected materials. We have thus had to make cuttings, root them in the greenhouse and then force new growth under 'clean' conditions. In those situations, one can achieve nearly 100% non-infected or disinfested cultures. Even so, because the Chinese tallow materials under study derive from open-pollinated seedlings, they are highly heterozygous. The variation in responsiveness among various (and vegetatively) cloned trees has been tremendous. Some have responded quite well, whereas others have been poor responders (figure 2 of Krikorian and Kann 1987).

On occasion I like to think that the variation in responsiveness is due to a sort of '*in vitro* management' deficiency on our part. This follows from an acute appreciation (that most experienced workers share) that there are various growth modes that can be fostered at will and maintained under aseptic culture conditions. When we elect to follow rigidly a particular strategy in multiplication, we probably preclude at least a few opportunities to learn the breadth of responsiveness of the system. It may well be that various individuals might be better responders under one set of conditions versus another, or as a result of one strategy versus another.

Also, part of the disadvantage or 'price one pays' of using, too early on the 'learning curve', a 'production mentality' that often dictates one should use (for reasons of economy etc.) a single medium for all individuals of a given species in that one tends to ignore what 'the system is trying to tell us' about its growth characteristics *in vitro*. In the 17th century, Nehemiah Grew, the father of plant anatomy in the English speaking world, said we must 'woo mother nature'. To now think that we can, at the outset, impose management regimes on *in vitro* systems is a delusion. I, myself, have still not come to any firm conclusions about what are being called 'consensus media'. These media are viewed as good for quite a few individuals, albeit not all. If the response is sufficient for a given purpose, then it can be adopted. Those individuals of a species which do not respond, don't matter, as it were. They 'fall through the cracks' (Christianson and Warnick 1985).

One should not, however, make the mistake of thinking failure to induce precocious branching, or shoots or roots on or from an explant is a 'management problem'. It is a basic science problem because we imperfectly understand the control mechanisms that relate to root initiation and formation, and growth, and bud and shoot development.

12. Culture-associated or somaclonal variation

No-one needs to be reminded that there are serious questions about the trueness-to-type of materials generated via various *in vitro* culture techniques. The situation involving aberrations in the flowering process in certain, nominally clonal, oil palm plantings in Malaysia has emphasized that one is still essentially at the basic research stage and not yet at the production stage (Corley *et al* 1986). It is apparently unclear exactly what went wrong in the process but it has been suggested that progeny of embryogenic cultures of oil palm which were still rather new in terms of their duration *in vitro* have been clonally stable (e. g. Paranjothy 1987). But, as the length of the aseptic culture period during which somatic embryos were replicated or multiplied became extended, the regenerates from these old cultures deteriorated in their performance. It is well-known that older cultures may decline in their ability to yield true-to-type progeny (Krikorian *et al* 1983). I hasten to add, however, that this is not tied to age of the culture alone (Krikorian 1988a, b, c).

The distressing feature of the oil palm fiasco to me, however, is that vegetative growth in the 'off-types' had been described as perfectly normal and only with the onset of flowering did the undesired variation show up. This means, of course, that unless one can guarantee clonal fidelity, the tissue culturing of oil palm for multiplication purposes is reduced to (elevated to?) an academic (and very important, I might add) exercise. None but the most research minded and wealthy grower is going to assume very readily the risk of raising *in vitro*-generated plants for several years only to discover that as the trees come into bearing age that their flowering biology is aberrant, thus jeopardizing the harvest!

Whatever the cause(s) of the abnormalities ultimately turn(s) out to be (Krikorian 1988a, b), I feel it would be instructive to be precise in describing what is meant by the relationship of clonal fidelity (or more accurately in this case, clonal fidelity of floral or reproductive biology) and duration of culture maintenance *in vitro*. The duration in culture must be qualified by the rate of cell division or the number of cell division cycles that the 'stem line' of the regenerative material underwent. If a slow growing regeneration system—embryogenic or otherwise—is sustained in culture for a long period, clearly the implication of this for occurrence of change of any sort in it will be different from a regeneration system that is fast growing and the number and rate of cell divisions is rapid. In the strictest use of the description 'duration in culture', one must also have information on the details of the regeneration including the cell biology of the mode (D'Amato 1986). Similarly, 'zero growth' or low growth or maintenance level growth can mean different things.

13. Possible causes of somaclonal variation

The number of possibilities as to what might go astray in a given culture system is great. In table 5 I have enumerated some of the possibilities. There is no pretense as to the completeness of this listing (Pearson 1968; Siminovitch 1976; McClintock 1978; Larkin and Snowcroft 1981; Burr and Burr 1981; Earle and DeMarly 1982; Krikorian *et al* 1983; Walbot and Cullis 1985; Snowcroft 1985; Karp and Bright 1985; D'Amato 1986 and references there cited).

All this emphasizes that until we learn to control cultures fully, one will not be in a strong position to use tissue culture for clonal multiplication of such things as

Table 5. Possible causes of somaclonal variation.

- Specific karyotype selection from mosaic, chimeric and polysomatic tissues and plants
- Karyotype changes due to differential response to culture procedures (media composition and/or environment)
  - —non-disjunctional aneuploides in culture
  - —mitotic arrest leading to polyploid lines
- Somatic segregation, gene rearrangements or mutations of the karyotype
- Gene amplification or diminution
- Virus elimination from selected lines of a culture
- Rearrangements or mutations in organellar genomes
- Altered nucleo-cytoplasmic interactions resulting in regulation changes
- Sudden reorganization of the genome by transposable elements
- Variegated position effects of chromosomal rearrangements (inversions, translocations etc.)
- Late replication of heterochromatin

horticultural or plantation crops or rigorously managed plantings of forest species unless we establish experimentally that a given protocol is reliable (Krikorian *et al* 1981; Karp and Bright 1985; Snowcroft 1985). In plantation crops, which generally have very stringent agronomic characteristics, any constraints or undesirable variations imposed on the qualities or features of the crop spell disaster. Dessert bananas grown in central and Latin America for export to north America or Europe are a good example of such a plantation crop. The management of the plantations is so tightly controlled and orchestrated that there are few degrees of freedom. The acceptable limits of deviation from the 'norm' are well-defined and if, for one reason or another there is a change, it is cause for alarm (Stover 1982; Rowe 1984; Stover and Buddenhagen 1986; Krikorian 1988a, b).

Recently, there has been some concern in the export banana industry, especially in the western hemisphere (but also in Taiwan, Australia, Israel and west Africa), over the high degree of lack of fidelity in certain tissue culture multiplied clones (Stover and Buddenhagen 1986). Plantings in Jamaica, derived from a commercial laboratory in the US, yielded very many (25%!) off-types (The Daily Gleaner, Kingston 7 October 1986). Many of these off-types, like those in the oil palm, became particularly conspicuous at the flowering stage. The plants were derived from highly organized shoot tip cultures (Oglesby and Griffis 1986). Whether the off-types arose as a result of what one might designate a poor tissue culture system management scheme, error in judgement during selection or from other reasons related to such phenomena as listed in table 5 is not known (Krikorian 1988a, b). A cooking banana clone (*Musa* ABB, 'Cardaba') multiplied in my laboratory at Stony Brook, reared to maturity in Puerto Rico with the collaboration of Dr Heber Irizarry and co-workers and rigorously evaluated, shows high clonal fidelity (unpublished results). One has to be very cautious, therefore, in drawing broad and sweeping conclusions of tissue-culture generated plants of any species. There are clear indications that some *Musa* clones show a rather high degree of clonal stability after *in vitro* regeneration/multiplication; others, like 'Grande Naine', a commercially important dessert banana of importance in the export trade have tended thus far to show much instability. One would, of course, like to know what renders one clone unstable and another less so. It has been suggested to me that 'Grande Naine' represents a clone that tends to show more instability even when multiplied via conventional agronomic means. But there are reports on 'Grande

Naine' being multiplied *in vitro* with nominally very high levels of fidelity (Arias and Valverde 1987). One could argue that it is due to the methodology employed or the material. Indeed, there is in the case of the banana clone 'Grande Naine', as there is in the case of all 'clones' of any vegetatively propagated plant, considerable range of phenotype. No two populations of 'Grande Naine' will be precisely identical when encountered in different locations.

All this underscores the level of our misconceptions and ignorance. The 'conventional wisdom' has been that virtually all plant materials generated from shoot tips or highly organized propagules, if you will, are stable. Strawberries are a good example of an exception to this statement (Marcotrigiano *et al* 1987). Materials generated via callus, cell suspensions etc have a much higher tendency to show culture associated variation. In the case of bananas and plantains, knowing what I did about the evolution and clonal nature of the genus, I took a conservative stance at the outset of our studies and predicted that change—even from shoot tips and meristems—could be anticipated. None really believed us—this is a good example of what I call 'most people hearing what they want to hear' (Krikorian and Cronauer 1984). It again becomes abundantly obvious that we do not have the fully reliable and firm access to the 'technology' that some have claimed us to have. In view of examples of the sort just given, one even seriously wonders if tissue culture can be considered a bona fide 'tool'—even in the so-called easy situations. It may be a 'device', it may be a 'means', it is certainly a 'procedure' that can be investigated. Not much more—yet! If we persist, one day it will be.

The argument that tissue culture-associated variation may be a great boon to agriculture (Larkin and Snowcroft 1981; Snowcroft 1984; Evans and Sharp 1988) is more than likely a red-herring and looks better on initial observation than on careful analysis (Sybing 1983). If one were to attempt an inventory of materials so generated that have made a positive and substantive impact at the practical level, one would be hard pressed. To the contrary, the variants or 'somaclones' are frequently completely worthless. On the other hand, I think that the effective study of the phenomenon(a) is crucial to our understanding of growth controls and development. The key(s), if indeed there is a single key, to knowing those mechanisms that confer or control plasticity in plant phenotype and genotype (Jennings and Trewavas 1986) may well be accessible through the study of somaclones.

One may well ask whether the problem of somaclonal variation has the same implications in the instance of tropical and subtropical forest species that it has in a rigorously managed tropical plantation crop comprised of, say, dessert bananas for export, rubber trees or even oil palm. My feeling is that it is perhaps not quite as important—certainly not in the early stages of research where one is just learning how to grow materials *in vitro*.

Whereas one can identify elite individuals or at least individuals with highly desirable qualities within a relatively densely grown population of an exotic commodity or foresty species such as *Eucalyptus* or *Acacia* etc. (Hartney 1980; Shepherd 1986), it becomes more difficult to do so within a stand of trees growing, say, in a tropical rain forest. They may not—indeed, they most likely will not—be growing in a 'stand' in the first place. They will be spread out, often growing substantial distances from one another. Features of growth may not easily be discernible or distinguishable because of microecological variables. Flowering patterns or floral biology may not be accessible and the practice of felling a very tall tree to collect herbarium specimens, once commonplace among field botanists, is largely a thing of the past. Sharpshooters who can retrieve precise samplings are not readily available. In short, in many cases we do not know what an elite specimen might comprise, so it becomes a moot point in the initial tissue culture process.

It may indeed turn out to be a 'blessing in disguise' for those seeking to work on the tissue culture of rather rare or uncommon tropical and subtropical forest trees, or those of commercial value for their timber etc. but which have not been reduced or 'elevated' to controlled forestry planting practices (Douglas 1972a, b, c). Since the field is wide open, and we are at the earliest of stages of dealing with these species *in vitro*, it serves no useful purpose to carp about the problems inherent in the tissue culture process of forest species. The recommendation would seem to be to learn all that one can about the species and then worry about problems a given system might pose to those wishing to use tissue culture for production purposes. Arguing the pros and cons of large scale clonal plantings is, to me, a bit like 'putting the cart before the horse' (McKeand and Weir 1984; Bey *et al* 1986a, b).

One feature of the tissue culture work that has been done thus far on many woody species, especially tropical forest trees, is that the potential strategies outlined in table 4 for use by tissue culturists become a largely theoretical issue. Rather than adopting a particular approach for multiplication, such as micropropagation via forced or precocious axillary branching (a method adopted in my laboratory for multiplying *Cinchona*, *Sapium*, *Vaccinium*, *Euphorbia*, *Hevea*, etc.), one sees a predominance of strategies or devices that reflect the fact that one follows a, or the route of multiplication that one is able to use. In a word, the response of the biological system may 'drive the approach'; the approach does not determine or impose on the plant, a given response. In those cases where one has a better idea of what the range of responsiveness is, one can then 'backtrack' and determine through research a strategy of choice. We often 'take what we can get', at this stage of our science (Bonga and Durzan 1987a, b, c).

It may also be that a given approach has a greater inherent risk for generation of culture-associated variants (George and Sherrington 1984). From my perspective, this is still far from being settled and probably never will be. This is because of the very fact that different plants may respond differently. Also, now that workers are more attuned to the phenomenon of somaclonal variation, there is much more awareness and desire to compare, rigorously, tissue culture-derived progeny with the starting material (Krikorian *et al* 1981; Evans *et al* 1984; D'Amato 1986 and references there cited). The fact that *in vitro* generated clones are being looked at more carefully than clones generated by non-*in vitro* means have ever been examined, seems to imply that some are hoping to elicit stability responses from tissue-cultured materials at a level greater than that achieved by mother nature. Similarly, the level of stability achieved often becomes a problem of interpretation unless one compares 'clones' generated by *in vitro* and non-*in vitro* means. From a practical perspective this becomes a 'catch 22' since the very purpose of cloning an élite or isolated, frequently unique, specimen is the very fact that it is in limited supply. One does not know the level of expected variation within the original clone. Intraclonal variation has long been recognized (Jackson *et al* 1985).

One feature of agri-forestry species, tropical timber trees and other woody species destined or projected, or at least valued for potential large-scale plantings, that imposes a particular perspective for me is the following. If truly large plantings are going to be needed, however far off in the future, as indeed I and many others believe to be the case, then the approach or strategy, if you will, of choice will necessarily implicate suspension cultures that are capable of yielding somatic embryos or related propagules (Steward and Krikorian 1975, 1979; Durzan 1980; Krikorian 1982). No other means that I am aware of can fulfill this requirement of large numbers in a timely and cost-effective manner. Also, if one anticipated ever being able to push basic, and eventually, applied, research on such woody species to its logical, maximal level (whatever that turns out to be), cells and protoplasts must perforce play an essential role in the process (figure 1). One simply has to recognize that one will have to at least evaluate the potential of this strategy (Hakman *et al* 1985; Tulecke and McGranahan 1985; Gupta and Durzan 1987).

In my laboratory, such motives and prejudices have constrained us to work, over many years, with cells in suspension of several monocotyledonous species that interest us (Krikorian 1982; Krikorian *et al* 1986; Krikoria and Kann 1986a, b; Krikorian *et al* 1988a, b). Our work on daylily (*Hemerocallis*) has shown that suspension-generated plants can have a high degree of clonal stability; protoplast-generated plants are, however, of much higher variability (Fitter and Krikorian 1988). I have no doubts, however, that other species might not follow that pattern.

14. Biology of explant source

The strategies enumerated in table 4, necessarily have consequences for the choice of starting material. The regrettable fact is, however, that we frequently have very poor, in some cases virtually no, information on the basic biology—physiology, biochemistry, anatomy, morphology and genetics of tropical and subtropical woody species. This is not only true of forest species but the statement is distressingly applicable to tropical tree fruit species as well (Ferwerda and Wit 1969; Page 1984; Bose 1985; Morton 1987 and references there cited). In addition to the shortage of information, one has also to deal with the perennial problem of limited accessibility to very good information on temperate trees (Schaffalitzky de Muckadel 1959). Clearly, an appreciation, if not an understanding, of biology and growth habits is critical to any informed decision-making, especially as to time and site of explantation (e.g. Wycherley 1973). Even the range of diversity of form is staggering (Halle and Oldeman 1975; Halle *et al* 1978; Fisher 1986). Some might assert that we are not necessarily that much better off in the case of temperate species but some excellent reference resources are available at least with reference to the classical literature (e.g. references cited in Matthews 1976). Also, new journals dedicated to woody species are emerging—e.g. *Trees*, Springer Verlag, vol. 1, 1987.

It will be like 'preaching to the converted', I hope, if I deplore the fact that we are frequently attempting to multiply and manipulate *in vitro* systems that we know all too little about *ex vitro* (Garner *et al* 1976; Macdonald 1986). Be that as it may, one can argue that all we can do is to try to make progress. At the same time, drawing attention to the facts of the situation might convince bureaucrats to recognize the need for supporting a broadly based programme of basic, botanical science. Tissue culture or 'biotechnology' cannot replace the dearth of basic information; it can only suffer from the lack of such baseline data. Indeed, it is dependent on it.

15. How much of a gap is there between knowledge and application?

The foregoing underscores the view that we definitely need more basic information on the systems that we seek to work on. On the other hand, we have a situation in which we do not always utilize that information which is available and could be drawn upon. Part of this, as stated, is due to our attitudes and inappropriate outlook on the entire process of plant tissue culture; part of it is due to an information block or lack of awareness of what is going on at the forefront of the field (Hutchinson and Zimmerman 1987).

16. Explant origin

The range of explantation sites available to the researcher is very large (table 4). When an investigator initiates a study it is a good approach to try a broad sampling of materials. In the first instance, the ones tried might well be determined by availability. Even in regions where a particular species is endemic, it may not be all that convenient to get specific material at will. While working with seedlings and juvenile materials is not to be preferred, it might be advantageous to learn how to approach a system using such materials. It can provide an idea as to what one might be able to expect of a species in terms of ease of obtaining disinfested materials, level of responsiveness etc. By the same token, one should as soon as possible seek to extend findings obtained with seedlings and juvenile materials to choice materials (assuming these are in relatively large supply). In general, one expects to achieve a more responsive situation in younger material. But the concept of juvenility can be extended to mature individuals as well, and should be.

Juvenility and age gradients in a regularly shaped (excurrent) conifer and a deciduous hardwood species are shown in figure 4. In the conifer, the *a b* axis represents youngest to most mature respectively. The laterals are shorter than the internodes they originated from; the distance *ab* is greater than *ac*, and *ac* is greater than *ad*, and *ad* is greater than *ae*, and *ae* is greater than *af*. Thus *b* is the most juvenile meristem shown. In the hardwood, density of cross hatching indicates the level of juvenility. Epicormics (branches developing from dormant buds on the trunk), spheroblasts (wood balls from dormant eyes disconnected from their vascular bundles), stump sprouts and severely pruned trees are all juvenile. Obtuse branch angles, single trunk and retention of leaves close to the trunk in winter also characterize juvenility (Zimmerman 1976; Bonga 1982).

In order to take advantage of an understanding of growth habit and patterns of maturation, it is essential that the person intending to carry out the tissue culture go to the field or forest with those most informed on matters of growth and development of the plant. All too often, the laboratory scientists fail to examine their materials as they grow under their natural habitats or plantations conditions. This deprives them of a first hand awareness of any distinctive phasic patterns of growth, features of habit such as orthotropy, plagiotropy (e.g. Krikorian 1985), features of environmental requirements (e.g. bright, direct versus indirect, subdued bright; soil pH; humidity; moisture requirements, etc.). Asking field-oriented personnel and colleagues who do not know anything about plant tissue culture to collect starting materials without prior detailed discussion is a mistake. It is critical to call upon them for help but one should at least initially go to the field with them.

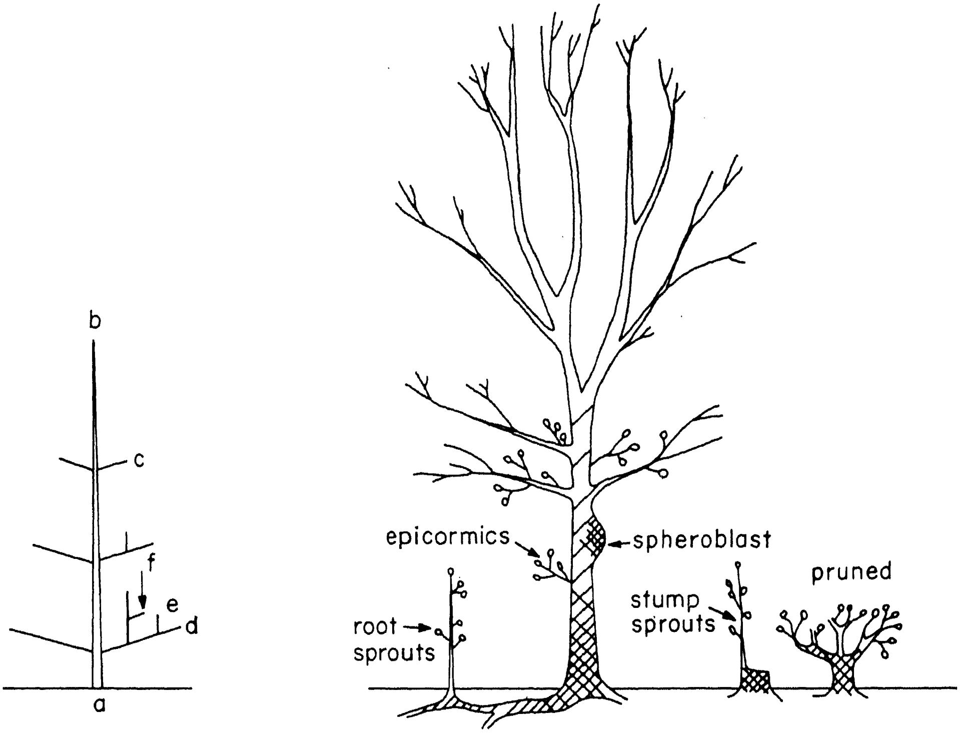

Figure 4. Juvenility and age gradients in a regularly shaped (excurrent) conifer and a deciduous hardwood species of decurrent habit. Redrawn with permission from Bonga (1982). See text for details.

I have been disappointed to see materials repeatedly placed *in vitro* with little regard to precise origin or developmental stage. Precautions should be taken at the outset to note the site of explantation along an axis; notation should be made as to distal or proximal end as well (acropetal or basipetal segments are rather easy to denote by the angle of cut such as accurately transverse versus oblique etc.). Horizontal position of an explant in or on the support medium where applicable—in, on or partially submerged in the medium; stuck upright or inverted in the agar etc.—all should be tested until information is obtained that it does or does not matter. The same holds for season or time of year, month and day for explantation. Even such seemingly obscure matters as culture vessel size, ratio of explant number to medium volume, symmetry of placement in a vessel etc. need to be noted. If an explant is touching the wall of a flask, for instance, this may stimulate ethylene production. The literature is replete with evidence (much of it not stated as such) that in certain instances each of these features is very important to the successful culture process.

Sources of explants currently viewed as somewhat unconventional should be tried as well. It has been known for quite a long time that certain species—including those of woody plants, exhibit the phenomenon of polyembryony (Gustafsson 1946). One of the classic examples is nucellus-derived somatic embryos of *Citrus* (Rangaswamy 1961). Mangosteen, *Garcinia mangostana*, was recognized long ago as asexually producing 'seed' as well and thus essentially cloning itself (Horn 1940). The literature is growing on the number of species that can be stimulated to form somatic embryos from explanted ovular or nucellar tissue (Litz *et al* 1985). While it is true that one is in most cases merely enhancing by *in vitro* means a naturally occurring phenomenon, the fact remains that such studies can only increase our understanding of underlying control mechanisms (Hanna 1987). It can also provide materials with which to study transition from juvenile to mature forms and related phenomena like topophysis (Zimmerman 1976).

Using roots as a source of explants is another approach which is too infrequently attempted. Isolated shoot tip explants which are rooted *in vitro* can provide roots for excision and manipulation. As is well known, shoot buds may exist on roots but are restrained from growth by correlative influences (Dostal 1967; Nozeran 1978). Even cases can be documented where no shoot primordia are known to exist in the root but can be stimulated *in vitro* by use of growth regulators etc. The oil palm is a good example of this (Krikorian and Kann 1986a, b). Clearly we need more information on the basic phenomenon of root formation and growth (e.g. Thornley 1977).

Another point that I should like to make is that very often we have inadequate information on the rooting ability of a given species, or not infrequently, we have misconceptions on how difficult it really is to get roots on conventional cuttings *ex vitro*. I have personally encountered more than a few situations in which it has been stated that rooting was very difficult, if not impossible. After further inquiry, it surfaced that no growth regulators or bottom heat or misting or fogging or anything else that one could call a 'state of the art' conventional horticultural propagational tool had been tested simply because they were not available (Garner *et al* 1976; Hartmann and Kester 1983). Leaves also can provide starting materials for multiplication operations. Too little attention has been paid to this potential with respect to woody species (e.g. Hagemann 1932; Billings *et al* 1988). In the case of *Coffea*, somatic embryos have been generated from leaf explants (e.g. Michaux-Ferriere *et al* 1987).

One might, in other cases, be resorting to plant tissue culture micropropagational strategies when they are not warranted because of disinformation or misinformation.

17. Growth regulators

Plant tissue culture workers are frequently preoccupied with added growth regulators—not only in terms of their identity but their level (Reynolds 1987). The early and important finding by Skoog and Miller (1957) that auxin; cytokinin ratios could determine whether shoots or roots formed in a given situation has dominated the thinking of most workers for too long, in my view, but there are signs that things are changing. Too many plants have not yielded to the manipulations that one would have prophesied on the basis of a control mechanism based directly on auxin:cytokinin ratio. The less narrow view which placed emphasis on the multi-component and multi-variate aspects of chemical plant growth regulation put forward (Steward and Krikorian 1971) was largely rejected because it could not be dealt with simplistically but it has now begun to be better appreciated. While more is being learned about growth regulators every day

(Davies 1987), we still have far to go to have a complete story. Lynn *et al* (1987) have isolated and identified a new class of growth regulators, dehydroconiferyl glucosides—DCGs that are related to lignins. These seem to be controlled by cytokinins and are to be seen as being at least a step closer to a controlling mechanism for cell division (Binns *et al* 1987). A major frustration for the person doing tissue culture is to have to deal empirically with each new situation. It is also becoming increasingly clear that one can do a great deal without exogenous growth regulators (Smith and Krikorian 1988).

Nevertheless, there are certain 'tricks of the trade' that are gained through experience. Use of Latin square experimental designs in which one varies two components, say auxin versus cytokinin, have been a mainstay of tissue culture methodology. But it can be useful in certain cases to try multiple auxins and multiple cytokinins. This device tacitly acknowledges that the presumed receptors or sites of action may not be exactly the same for each class of chemical substance. There is evidence that combinations can bring about better responses than substances used singly. Quantities of several chemicals that are equivalent in molar amount to that used of a single substance may yield a response far in excess of what one might get with the one alone. Synergism is a phenomenon that is not well understood (Krikorian *et al* 1987).

17. Adjuvants and modulators

While one can easily become engrossed in the matter of cytokinins and auxins, there are a host of other substances that can influence and modulate *in vitro* growth responses (Steward and Krikorian 1971; Reynolds 1987; Krikorian *et al* 1987).

18. Activated charcoal

Activated charcoal has been used for many years as an adsorbent of presumably toxic chemicals in the growing of plants. Epiphytic orchid growers have long relied on charcoal chips as an additive to their substrates. The earliest use of activated charcoal in an aseptic tissue culture medium that I have been able to track down relates to use with lower plants (Proskauer and Berman 1970) and it was also extended to orchids around this time (Werckmeister 1971). Activated charcoal can provide a darkened environment for growth of roots but since it is 'activated', it does things additional to merely darkening the medium. Some years ago in my laboratory, Dr Madhav Singh of Department of Biochemistry, Lucknow University and I decided to check the impact of providing cultures a darkened root environment versus a darkened and activated one. Lamp black was purchased and exhaustively washed with petroleum ether in a Soxhlet apparatus to get rid of toxic components and then washed with water and dried at a relatively low temperature. Activated charcoal was washed and re-activated with HCl and dried at activating temperatures to provide a source of freshly and uniformly activated source of 'activated charcoal'. The data obtained, which will not be presented here, indicated that in some cases mere darkening of the medium was sufficient to generate a healthy crop of roots. If activated charcoal was used, the level of auxin (or other growth regulator) had to be increased up to 100-fold since it was being adsorbed and thus not becoming as readily available to the tissue being stimulated as it would be in the absence of the adsorbent.

What I am saying is that one can conserve growth regulators in some instances such as rooting experiments by using media darkened with non-adsorbent materials like non-activated but rigorously cleaned lampblack. Wrapping aluminum foil around the bottom of a tube to 'darken' the roots is generally inadequate since the surface of the agar medium is still exposed to light. In fact, it may do just the opposite since with partial obscurity, one is creating a lens effect.

In other instances, the use of activated charcoal can make a major difference in the success or failure of a given culture attempt (Bon *et al* 1988). Activated charcoal does adsorb growth regulators but it also adsorbs substances presumed to be injurious like certain phenolics, oxidized polyphenols, cell lysis products etc. Gases like ethylene and methane likewise thought by some to be counter-productive to tissue culture efforts can also be adsorbed. We again know all too little about the role of controlled release of growth regulators or adsorption of metabolic by-products to make generalizations. One has to run controlled experiments to see what the effects are in a given case.

Having said the above I will interject here that it is important in a controlled experiment to use properly activated charcoal. Too many workers rely on brand name sources of activated charcoal. The fact is that activated or decolorizing carbon becomes deactivated with time and in order to have uniformly activated stuff one should carry out re-activation in the laboratory. This is achievable by several means but one approach is to expose (via refluxing in a fume hood) the charcoal with hot 2 N HCl (preferably in a Soxhlet apparatus using an acid resistant 'thimble'—but a beaker with glass boiling chips will do), exhaustively rinse with water to get rid of excess Cl^- (check with $AgNO_3$ solution to the point where there is no precipitation of AgCl noted) and dry in an oven at around 110°C for several hours to completely dry. Heating also drives off any oxygen that occupies the sites available to adsorption. The charcoal can thus be stored in a desiccator and used as necessary*. Activating a quantity sufficient to last a year is not a great inconvenience and eliminates any doubts as to level of activation if charcoal is used. Although one reads and hears of the superiority of one brand over another, it has no defensible basis in fact. There is a large literature on activated charcoal that is available to all (Houghton and Wildman 1971; Dandy 1977).

19. Phenolics and other adjuvants

Phenolics frequently have the connotation of substances to be avoided since in the oxidized form they are perceived as inhibitory to growth. Primary explants of woody species, especially tropical and subtropical woody species, seem to be very prone to darkening *in vitro* and it has been assumed that this is the direct result of stress and polyphenol oxidase-induced chemical reactions (e.g. Goodman *et al*

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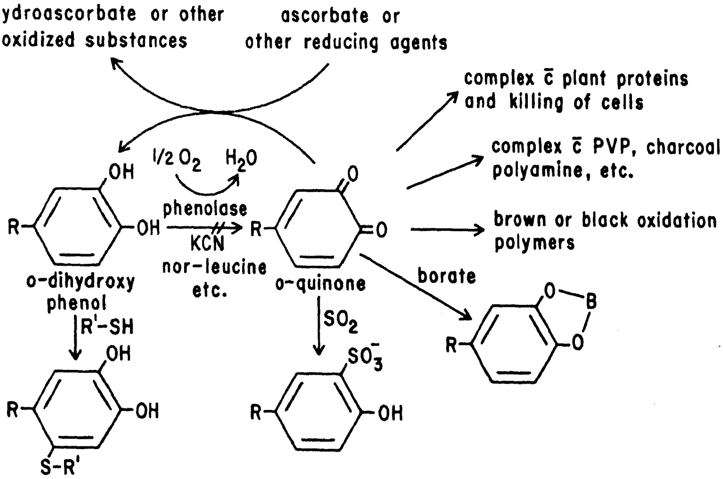
*For 20 g of charcoal to be reactivated, use 250–300 ml 2 N HCl in a 500–600 ml beaker and heat to boiling with continuous stirring. Boil for 3–4 h in a fume hood. Cool to room temperature and wash with distilled water until Cl^- free on a Buchner funnel. Warm distilled water ($\simeq 50°C$ or so) removes Cl^- more easily. Ten to 20 litres of water may be necessary for washing. When using nominally activated charcoal that has been activated sometime earlier, heat first at 110°C for an hour or so.

1967; McClure 1979 and references there cited). The oxidation products of polyphenols are seen as normal responses to insect and fungal attack and injury-related responses etc. (Uritani 1978; Vaughn and Duke 1984).

Whether extra-ordinary attempts should be made to minimize, counteract or otherwise eliminate the blackening or darkening responses is a matter that is fraught with much controversy (figure 5). No doubt that as many opinions could be elicited as persons surveyed. In my view, use of antioxidants can help a bit but they rarely, if ever, make the difference between success and failure.

If one subscribes to the view that the production of black or brown substances by cultures *in vitro* is a reflection of a normal response mechanism being incited by the culture process in species capable of producing phenolics in quantity, several alternatives present themselves. (i) Ignore the darkening and ascertain whether some of the tissues are adversely affected or not. Plenty of tissues which initially appear very dark brown or black can grow very well. (ii) If the darkened tissues do not grow, ascertain whether sectors of the culture remain unaffected and do not blacken or darken. Selection of non-darkened tissue capable of growing can be carried out and stocks thus can be built up. (iii) Another strategy to be adopted involves frequently—as frequent as daily—carrying out transfers to fresh medium to eliminate or at least minimize accumulation of presumed toxic substances. If the conditions for growth are permissive, then one might, in effect, outpace the darkening phenomenon and/or 'turn it off'. (iv) Turning off the darkening phenomenon can on occasion be achieved by driving the reaction to the right or left by deliberately providing an abundance of precursor or the oxidized product. (v) Lowering of copper (Cu^{+2}), the co-factor for polyphenol oxidase, in the medium by omitting copper sulphate from media recipe can be helpful. There is plenty of copper in a medium since it is a ubiquitous trace contaminant and omitting it will usually not cause deficiency. (vi) A prolonged period of culture in lowered or total absence of light can favor decrease of darkening (e.g. Zaprometov 1978). One



dehydroascorbate or other oxidized substances
ascorbate or other reducing agents
complex c̄ plant proteins and killing of cells
complex c̄ PVP, charcoal, polyamine, etc.
brown or black oxidation polymers
1/2 O2
H2O
phenolase
KCN
nor-leucine etc.
OH
R
o-dihydroxy phenol
o-quinone
O
borate
B
R'-SH
S-R'
SO2
SO3−

Figure 5. Scheme showing some relationships between phenolics, their oxidation to yield darkened products, and intervention in their oxidation with various reducing and adsorbing agents.

cannot generalize but darkness generally brings about an increase in the level of phenolics whereas light does the opposite. The role of phenolics is very complex and the value of the addition of these to a given culture medium should be determined with care.

There are a number of additional strategies that have some usefulness. Reducing agent 'washes' comprised of ascorbic acid, citric acid and/or cysteine have been said to be helpful by some. Since they are heat labile, they should be prepared and used after filter sterilization. Substances like dithiothreitol (DTT), otherwise known as Cleland's reagent, are reportedly effective as well. Reuveni and Golubowicz (1983) have used a solution of 100 mg/l of DTT as an anti-oxidant while making cuts on date and banana tissue cultures. They also have used citric acid 2000 mg/l and ascorbic acid 150 mg/l as an alternative. In date palm tissue culture, caffeine, 2000 mg/l; sodium diethyldithiocarbamate 1000 mg/l; and polyvinylpyrollidone (PVP) 1000 mg/l has been used to keep sterilized material from darkening prior to final preparation for tissue culture. Other recipes call for 500 mg/l PVP and 250 mg/l ascorbic acid (Rhiss 1980). There are numerous substances that can be tested and added but one wonders if their use is justified. DTT is used as a protecting agent for sulfhydryl groups in the isolation of enzyme proteins and hence can be thought of as an agent capable of maintaining monothiols completely reduced and disulfides quantitatively reduced (Cleland 1964) (figure 6). Attempts to confirm this allegedly beneficial activity of DTT in my laboratory have not been successful (Ziv and Halevy 1983). Most of the other anti-darkening agents other than activated charcoal have likewise been adjudicated by us as virtually worthless. We have adopted therefore the view that darkening of the medium is not necessarily cause for major concern but if the explant darkens and does not grow we do have to worry and reconsider the culture strategy being adopted. Even here, however, as has been mentioned, some tissues darken and continue to grow vigorously. In our hands these kinds of tissues generally are of greater interest for the study of secondary product metabolism rather than for regeneration of plants (DiCosmo and Towers 1984; Constabel and Vasil 1987).

As yet another example of the level of decision making during the implementation of a tissue culture programme one can provide evidence that use of wetting agents in the disinfestation step can be harmful. Use of oxidizing agents such as hypochlorite is thought by some to be facilitated by addition of Tween 20 or 80, or wetting agents like Teepol etc., but one may in fact be activating the various phenol oxidase systems. Ethyl alcohol may in some instances be sufficient for external sterilization and in extreme cases, dipping in alcohol and flaming for very brief periods, extreme as it may sound, can be helpful (e.g. Bakry *et al* 1985).

Finally, all darkening encountered in *in vitro* situations is not enzyme associated. Non-enzymic browning has been a major concern of the food industry for many years (Hodge 1953; Reynolds 1963; Dworschak 1980). Whether these substances have a role to play in the growth of a given species *in vitro* is a matter that has to be ascertained case by case (Compton and Preece 1988).

20. Diversion from 'usual' strategies

The use of approaches that at the outset seem at variance with 'approved' practices can be of some use in dealing with recalcitrant cases. A few examples are given.

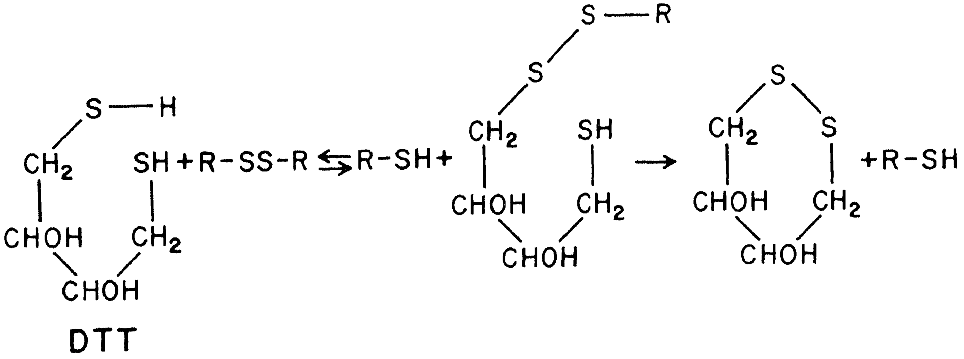


Figure 6. DTT as an anti-darkening agent may be tied to its reducing action upon disulfides.

Alternating an explant from semi-solid to liquid medium, back and forth, can 'push' a system that is sluggish (Cronauer and Krikorian 1984). The idea here is to alternate all those parameters associated with the status of being in or on a semi-solid medium with those of liquid—whether partially or wholly submerged. It can also be seen from the perspective of osmotic or nutritional shock.

Preparation of protoplasts in quantity and using these as a means of generating suspension or callus cultures is another approach that can be useful. This practice is based on the assumption that of the large number of cells that comprise an organ or tissue, only a limited number can at any one time be in a mode responsive to cell division and growth stimuli. By reducing the organ to its component cells, those which cannot respond are shocked into degeneration whereas those that can survive and grow. This is the equivalent of selection at the very early stage of culture rather than at the later stage(s) of culture (Krikorian *et al* 1988a, b).

21. Use of co-culture

Species that grow well and which have recognizable growth characteristics can be used to test whether they can nurture or otherwise hasten the growth responsiveness of a recalcitrant tissue. Conditioned media responses are generally somewhat inconclusive but are well worth trying in those cases where a system is really stubborn.

22. How to accelerate activities to improve both knowledge and to make positive contributions

Throughout this paper, the view has been adopted that one of the major failings in plant tissue culture is that there is a serious mismatch between perception and reality. This in turn frequently leads to disillusionment. Many have gotten the idea that all kinds of capability is in hand and one has only to initiate a project and it all falls in to place. After all, a biotechnology is nothing but a risk-free commodity which can be paid for and used.

It is critical to understand that plant tissue culture is not a technology in the conventional sense of the word. One must distinguish between research, development and production. By confusing the 3 and ignoring the critical element of milestones and timelines as well, we have confusion of what we can expect and the period of time in which we can expect it. Tissue culture is a process, not an event.

Most laboratories in the tropics and subtropics have adopted as their first goal, the controlled tissue culturing for purposes of production, some of the most challenging plants that I, personally, am aware of. There is frequently all too little evidence that the laboratories in question are operating effectively at baseline level. That is, if one were to adopt the goal of culturing an 'easy' plant like carrot or tobacco so as to ascertain that all components of the laboratory mechanics were optimally functional, one could perhaps feel more confident that those plant sytems generally adjudicated as 'difficult' are really so. After all, one needs a standard of comparison against which one can draw conclusions. Few laboratories I have encountered have demonstrated that they can do the easy stuff. Instead, they have jumped into the nominally difficult stuff. The negative impact of matters such as water quality, marginal cleanliness of glassware, etc. as deterrants to optimal growth will forever remain in question unless one demonstrates that these and similar problems are not sources of limitation.

Also, all too many investigators in the tropics and subtropics are overburdened with administrative and other duties. Very often, the responsibility of doing the day to day operations or the actual culture work devolves upon junior level people and/or technicians. When an investigator is not closely attuned to what is happening on a daily basis and is not, particularly throughout the early development phase of a project, aware of nuances of responsiveness of a given culture system, one is asking for trouble. This situation is often a direct reflection of the mistaken attitude that what one is doing is a technology. The scientist must be in charge. Only when the system is well on its way should one expect that technicians can carry the physical work load of the project.

From this, it logically flows that routine and daily inspections must be made of cultures to evaluate their progress. Obviously in cases where cultures are being grown in total darkness, one cannot do this but enough replicates should be made so as to allow a sacrifice of a few for regular examination. A hand lens of 10 or 20 × with a convenient working distance is a major asset to anyone making inspections.

I have seen time and again workers who expect cultures to 'wave coloured flags' when showing a positive response. Experience is a major advantage in this kind of work but one does not gain experience without carefully examining cultures and watching them grow. Setting up cultures and ignoring them for 3–4 weeks is not the way to gain experience! Undiplomatically stated, one must work and apply oneself in order to reap the benefits of *in vitro* work. One cannot get discouraged easily, and perhaps more important than anything, one has to enjoy growing things. The addage of the proverbial 'green thumb' versus the thumb seemingly imbued with the 'kiss of death' seems to have merit. Some individuals are just not cut out for tissue culture work. The better part of valour is to recognize it and seek one's fortunes elsewhere.

The inevitable conclusion seems to be that while woody plants, especially tropical and subtropical ones, are not the easiest species to work with, they are becoming increasingly tractable. As more and more capable people undertake studies on these important plants the situation will dramatically change. In the beginning period, it may well be pardonable to perform probes or tests, sometimes referred to as 'going on a fishing expedition', to see the level of responsiveness that can be elicited. If one gets an encouraging response, then one can 'backtrack' and perform a definitive experiment. Even in cases where there is a limited supply of material for primary explantation one can do some meaningful work. Appropriate numbers of replicates can be instituted later. While one obviously always seeks to do carefully controlled and replicated laboratory tests, it may be advisable in some select cases to get a result first and then to worry about 'how and why'. Also, one should not minimize the importance of the serendipity factor. 'Accidents' usually provide that critical impetus to science if one is informed and prepared to recognize the value of an important accidental finding. The production in profusion of somatic embryos by cultured carrot cells is a classic example of fortuitous finding that spawned a whole new approach to plant tissue culture (Krikorian and Berquam 1969; Krikorian 1988a, b).

There is an old and familiar saying that relates, 'nothing succeeds like success'. This is no doubt true but the means whereby one creates opportunities for oneself in plant tissue culture work are so numerous that the saying takes on even more significance. Plants, by virtue of their evolutionary origins, are highly plastic and seem to have multiple levels of control systems built into their very being. Perturbation of their normally directed and tightly controlled pathways of growth and development open up new and exciting chances to disclose how things work and what the controlling mechanisms might be. It will be a great pity if plant tissue culture workers become too closely attached to a given goal, such as the cloning of a species in large numbers, and fail to see what a given system is trying to tell them. Only by recognizing that there is much plasticity (Jennings and Trewavas 1986) and hence there are multiple routes and strategies, and that a given sequence of responses can be correlated with progressive increase or reduction in size etc., will we begin to define the causal parameters in such ways that they can be tested through experiments. If and when this happens, we will begin to produce the data base that will render plant tissue culture a true biotechnology.

Acknowledgements

The views expressed in this paper reflect experience gained since the time when he was a beginning graduate student at Cornell University in the laboratory of Professor F C Steward, F.R.S. Since then numerous grants, travel opportunities and exchanges with colleagues have provided the mechanism whereby much first-hand exposure has culminated in the position taken. Support from the NASA is particularly acknowledged since Dr Thora W Halstead has held the view that plant tissue culture work is an important means of studying control mechanisms and will have an increasingly important role to play not only in basic space flight experimentation but in efforts seeking to provide controlled ecological life support systems for extended duration space flights. Sustained support has given us the necessary continuity in our projects. US Agency for International Development has also supported some of our work as well as the US National Science Foundation, including the Division of International Programs. The broadly scoped work of many students, research associates and technicians has likewise provided the base of experience so necessary to a conviction of the sort expressed.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 6, December 1988, pp. 465–470.
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Oedogoniales of Uttar Pradesh, India—*Oedogonium* Link

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MS received 29 December 1987; revised 3 October 1988

Abstract. Seven species of *Oedogonium* Link collected from Mauri lake, Pratapgarh, Uttar Pradesh during July 1984 to August 1986. Of these *Oedogonium boscii* (Wittr.) Hirn var. *minor* var. nov. is new for Phycology; two species viz. *Oedogonium grande* (Kuetz.) Hirn and *Oedogonium praelongum* Hall. *ex* And. are first records for India and the remaining 4 species viz. *Oedogonium mexicanum* (Wittr.) Hirn, *Oedogonium exocostatum* Tiffany var. *jodhpurense* Goyal, *Oedogonium costatosporum* Jao var. *longisporum* Gonzalves *et* Sonnad and *Oedogonium epiphyticum* (Trans. *et* Tiffany) Tiffany are new records for northern part of India.

Keywords. Epiphytic; Chlorophyceae; Oedogoniales.

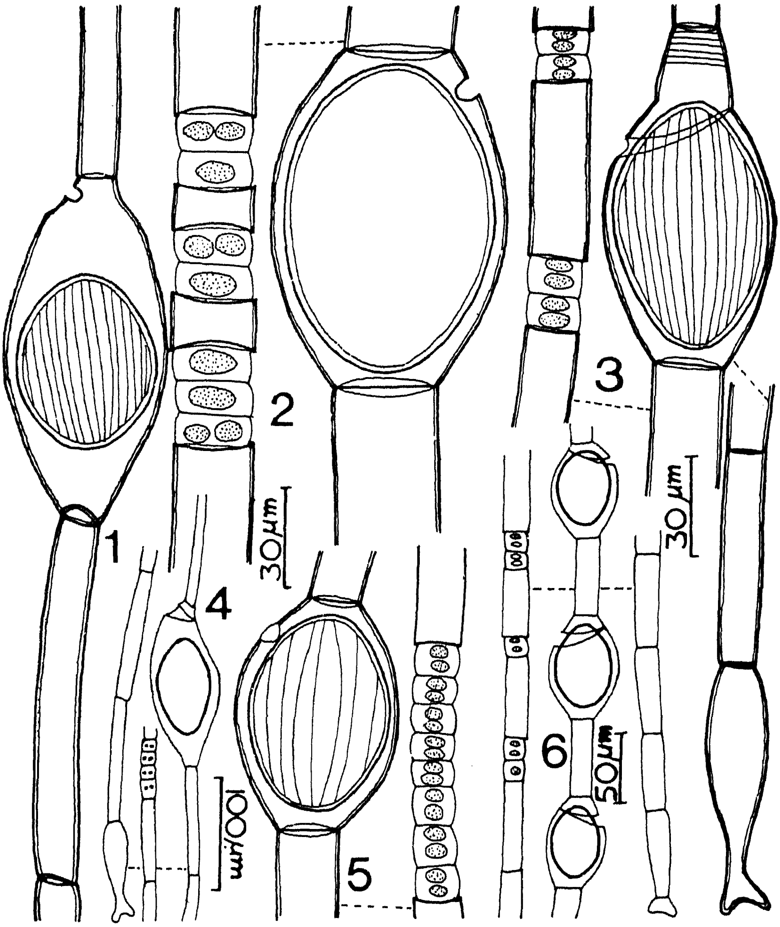
1. Introduction

The genus *Oedogonium* Link, is known to have 531 species (Hirn 1900; Gonzalves 1981) and out of which 310 species are known from India (Gonzalves 1981). So far only 77 species (macrandrous, monoecious) are reported from India and amongsts them 19 macrandrous, monoecious species have been recorded from Uttar Pradesh (Singh 1938, 1960; Venkataraman 1957, 1959; Singh 1959; Pandey 1965; Khan and Kukereti 1977). The present communication deals with 7 species of *Oedogonium*, of these *Oedogonium boscii* (Wittr.) Hirn var. *minor* var. nov. is new for phycology; two species viz. *Oedogonium grande* (Kuetz.) Hirn *Oedogonium praelongum* Hall. *ex* And. are first records from India and 4 species viz. *Oedogonium mexicanum* (Wittr.) Hirn, *Oedogonium exocostatum* Tiffany var. *jodhpurense* Goyal, *Oedogonium costatosporum* Jao var. *longisporum* Gonzalves *et* Sonnad and *Oedogonium epiphyticum* (Trans. *et* Tiffany) Tiffany are new records for northern part of India. All the 7 species were collected from Mauri lake, Pratapgarh, Uttar Pradesh during July 1984 to August 1986.

2. Taxonomic treatment

2.1 *Oedogonium boscii* (Wittrock) Hirn var. *minor* var nov. (figure 1)

Filamenta macrandra, dioecia; cellulae vegetativae cylindricae; cellulis filamentorum masculorum angustiribus quameis filamentae femineae; cellulis filamentorum femineorum 10–12 μm dia., 90–110 μm long; oogonia singula, ellipsoidea, 40–42 μm dia., 85–90 μm long; porus superior, oosporae sphericae, ovoideae, tribus stratis compositae, strato exteriore longitudinaliter costato, costae 20–25 numero, oosporeae oogonia non complentes, 35–38 μm dia., 42–48 μm long; antheridea



Figures 1–6. **1.** *Oedogonium boscii* (Wittrock) Hirn var. *minor* var. nov. **2.** *O. grande* (Kuetzing) Hirn. **3.** *O. costatosporum* Jao var. *longisporum* Gonzalves *et* Sonnad. **4.** *O. praelongum* Hall. *ex* And. **5.** *O. exocostatum* var. *jodhpurense* Goyal. **6.** *O. epiphyticum* (Trans. *et* Tiffany) Tiffany.

singula aut usque ad quinque, sparsa, 8–9 µm dia., 8–10 µm long; spermatozoidea duo, divisione verticali.

Locus typi: In loco Mauri lake, Pratapgarh, Uttar Pradesh, dicto. Tempus collectionis 10–12–84; Planta epiphytica in *Hydrilla verticillata.*

Specimen typicum: Algae HMS-2. Materia in departmento Botanco Universitatis Allahabad, Allahabad, India deposita.

Macrandrous, dioecious; vegetative cells cylindrical, cells of male filaments narrower, cells of female filaments 10–12 µm in dia., 90–110 µm in length; oogonia single, ellipsoidal, 40–42 µm in dia., 85–90 µm in length; poriferous, pores superior; oospores spherical, ovoid, 3 layered, outer layer longitudinally costate, costae 20–25 in number, not completely filling the lumen of oogonia, 35–38 µm in dia., 42–48 µm long; antheridia single or upto 5, scattered, 8–9 μm in diameter, 8–10 μm in length; spermatozoids two, division vertical.

Type locality: Mauri lake, Pratapgarh, Uttar Pradesh; collection date: 10–12–84; Epiphytic on *Hydrilla verticillata.*

Type specimen: Algae HMS-2. Material deposited in the Department of Botany, University of Allahabad, Allahabad.

Except smaller dimensions of vegetative cells, oogonia and oospores the present alga comes close to *Oedogonium boscii* because of the differences mentioned above it has been proposed as a new variety of the type species. The present alga does not resemble in measurements any other varieties and forma of the species.

2.2 *Oedogonium grande* (Kuetzing) Hirn (figure 2).
Gonzalves 1981, 272, figure 9. 151.

Macrandrous, dioecious; vegetative cells cylindrical, male filaments slightly narrower than cells of female filaments, cells (female filaments) 24–34 μm in dia., 130–200 μm in length; basal cell elongated; oogonia single or upto 4, subobovoid, 50–65 μm in dia., 80–100 μm long; poriferous, pore superior; oospores also subobovoid, smooth walled, completely filling the lumen of oogonia, 48–63 μm in dia., 78–85 μm long; anthridia single or upto 4, seriate, 18–22 μm in dia., 7–12 μm in length; spermatozoids two, division vertical.

The present alga has similarities in general appearance with the *Oedogonium crassum* (Wittr.) Hirn and *Oedogonium mexicanum* (Wittr.) Hirn, but the latter species have larger dimensions of vegetative cells and spherical to cylindrical obovoid shape of oogonia. The present alga well agrees with the type description.

Collection no.: 57, 126.

Habitat: Epiphytic on *Hydrilla verticillata* and *Aponogeton crispus.*

Occurrence: September to February.

Distribution: It appears that the species is the first report from India, though it has been reported earlier from other countries of the world viz. Africa, north America, Asia (China and Bangladesh), Europe, Oceania and West Indies (Gonzalves 1981).

2.3 *Oedogonium praelongum* Hall. *ex* And. (figure 4).
Gonzalves 1981, 345, figure 9. 230.

Macrandrous, dioecious; vegetative cells cylindrical, cells of female filaments slightly smaller than those of male filaments, cells (female) 7–11 μm in dia., 50–150 μm in length; basal cell elongated, 20–25 μm in dia., 80–90 μm in length; oogonia single, ellipsoidal, 35–44 μm in dia., 80–90 μm long; operculate, division superior; oospores ellipsoidal, spherical, smooth walled, not completely filling the lumen of the oogonia, 34–41 μm in diameter, 45–55 μm long; antheridia upto 18 in a series, 14–16 μm in dia., 11–15 μm long; spermatozoids two, division vertical.

The present alga agrees well with the type description.

Collection no.: 50, 181.

Habitat: Epiphytic on *Ipomoea nil* and *Chara zeylanica.*

Occurrence: October to March.

Distribution: As far as the author is aware this species has not been reported earlier from India. Though earlier it was recorded only from Europe (Gonzalves 1981).

2.4 *Oedogonium mexicanum* (Wittrock) Hirn.
Hirn 1900, 147, plate 22, figure 111; Gonzalves 1981, 284, figure 9.163.

The present alga resembles with the type description. The alga can be compared with *Oedogonium grande* (Kuetz.) Hirn, in its general appearance and in measurements. However, *Oedogonium mexicanum* is known to have characteristic cylindrical oogonia and their solitory occurrence.

Collection no.: 57, 220.

Habitat: Epiphytic on *Aponogeton crispus*, *Hydrilla verticillata* and *Cyanodon dactylon.*

Occurrence: October to March.

Distribution: Previously this species has been reported only from Ahmedabad. Gujarat (Kamat 1962).

2.5 *Oedogonium exocostatum* Tiffany var. *jodhpurense* Goyal (figure 5).
Goyal 1964, 389, plate 1, figures 16–17; Gonzalves 1981, 311, figure 9.186 B.

The present alga agrees well with the variety *jodhpurense* though differs from the type species, in having inflated suffultory cells and shorter dimensions of vegetative cells.

Collection no.: 56, 181.

Habitat: Epiphytic on *Ceratophyllum demersum*, *Neptunia oleracea* and *Chara zeylanica.*

Occurrence: October to January.

Distribution: First from northern part of India, earlier reported from Jodhpur, Rajasthan (Goyal 1964).

2.6 *Oedogonium costatosporum* Jao var. *longisporum* Gonzalves and Sonnad (figure 3).

Gonzalves and Sonnad 1961, 718, figures 6a–c; Gonzalves 1981, 354, figure 9.239 B.

The present alga resembles the type in all the essential characteristic features. It differs from the type species in having longer oogonia and oospores and also longer and narrower terminal cell.

Collection no.: 86, 194.

Habitat: Epiphytic on *Utricularia aurea* and *Najas graminea.*

Occurrence: November to February.

Distribution: Earlier recorded from Naglavi, Karnataka (Gonzalves and Sonnad 1961).

2.7 *Oedogonium epiphyticum* (Trans. *et* Tiffany) Tiffany (figure 6).
Gonzalves 1981, 338, figure 9.221.

The present alga agrees well with the type in all essential characters. But for slightly broader oogonia.

Collection no.: 68, 224.

Habitat: Epiphytic on *Aponogeton crispus, Cyanodon dactylon, Cladophora glomerata* and *Oedogonium princeps.*

Occurrence: November to March.

Distribution: Earlier it was reported from Devarai, Karnataka (Gonzalves and Sonnad 1961), Kolhapur, Maharashtra (Kamat 1963).

Acknowledgements

The authors are thankful to the late Prof. D C Pandey, Department of Botany, University of Allahabad, Allahabad, for kind help and suggestions and the late Dr H Croasdale, USA, for kindly rendering the Latin diagonosis and gratefully acknowledges the financial assistance from the University Grants Commission, New Delhi.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 6, December 1988, pp. 471–474.
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Leaf architecture in Tamaricaceae

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MS received 21 July 1987; revised 9 September 1988

Abstract. The leaf architectural pattern in 6 species of *Tamarix* and two species of *Myricaria* has been investigated. The leaves are simple, alternate and sessile. In *Tamarix aphylla*, *Tamarix dioica*, *Tamarix gracilis* and *Tamarix troupii* the leaves are completely or partially sheathing. In these species only the primary vein is distinguishable, the secondaries and the further orders of venation being not distinguishable from one another. In the other two species of *Tamarix* and in *Myricaria* the major venation pattern is pinnate-camptodromous type. The intercostal venation is of random reticulate type, and the marginal ultimate venation is incomplete. The areoles are polygonal to irregular in outline and randomly oriented. They may contain one or more veinlets or none. There is no direct correlation between the size of an areole and the number of vein endings. Hence, these characters cannot be said to be taxonomically significant as far as the species of *Tamarix* and *Myricaria* are concerned.

Keywords. Leaf architecture; Tamaricaceae.

1. Introduction

The use of leaf architectural patterns has been studied in many families so far. It is gaining significance in phylogeny and classification of angiosperms. Recently these characters have been successfully used by several workers in diagonosis of fossil as well as living material (see Dilcher 1974). Hickey (1973) presented a classification of the architecture of dicotyledonous leaves opening a new venue of taxonomic and morphological studies. In the family Tamaricaceae, the leaves are very small and scaly in nature, as majority of the members are xerophytic.

2. Materials and methods

The materials of *Tamarix aphylla* Karst., *T. dioica* Roxb. and *T. troupii* Hole were collected from Mathura and Ghaziabad districts, while those of *T. gracilis* Willd., *T. ramosissima* Ledeb., *T. smyrnensis* Bge and *Myricaria alopecuroides* Schrenk were sent from Moscow by Dr T Pullaiah. The material of *Myricaria germanica* Desv. was obtained from Prof. H Merker of Sweden.

For studying venation pattern the leaves were washed with water and kept in 10% KOH at 50–60°C in oven till the leaf got decoloured. Then the leaves were thoroughly washed with water and were transferred to saturated solution of chloral hydrate (prepared in 20–40 volume in hydrogen peroxide) till the leaves became transparent. Cleared leaves were stained in 1% basic fuchsin solution prepared in 90% ethanol and were mounted in Canada balsam.

**Since deceased.

3. Results

3.1 *Gross morphology*

The terminology followed here is after Hickey (1973) with certain modifications.

The leaves are simple, alternate and sessile. They are completely sheathing in *T. aphylla* and *T. dioica* and partially sheathing in *T. gracilis* and *T. troupii.*

The lamina is symmetrical in all the species examined. The shape of leaf is ovate in *T. aphylla*, *T. dioica*, *T. troupii* and *M. germanica*; narrow ovate in *T. gracilis*; linear-lanceolate in *T. smyrnensis* and oblong in *M. alopecuroides.* The apex is acute in *T. gracilis*, *T. ramosissima* and *T. smyrnensis*, acuminate in *T. aphylla*, *T. dioica* and *T. troupii* and obtuse in *M. alopecuroides* and *M. germanica.* The margin is entire in all the species examined. Salt glands are frequently present on both the surfaces of lamina.

3.2 *Venation pattern*

The venation is unicostate reticulate type. In *T. aphylla*, *T. dioica*, *T. gracilis* and *T. troupii*, only primary veins could be distinguished, the secondaries and the further order of veins are not distinguishable from one another (figure 1D, E). In the other species of *Tamarix* and *Myricaria* examined, the major venation pattern is pinnate-camptodromous type i.e. the secondary veins do not terminate at the margin (Hickey 1973).

There is a single primary vein (mid-vein) which runs straight upto the apex in all the species studied.

The veins of the second order in *T. ramosissima*, *T. smyrnensis*, *Myricaria alopecuroides* and *M. germanica* arise alternately on both the sides of the primary vein (figure 1A, B, C, F). The secondaries are straight at basal region and recurved at the apical region (figure 1B, C, F). The intersecondary veins could not be distinguished.

The next finer branches of the secondary veins and branches of equal thickness arising from primary, vein are the tertiary veins. The angle of divergence of tertiaries varies irregularly. They freely remify towards the margin and anastomose with other tertiary veins at various angles forming random reticulate pattern (figure 1A, B, C, F). Next higher vein orders could not be distinguished in any of the species examined.

The ultimate marginal venation is incomplete or imperfect (free veinlets endings are present directly adjacent to the margin) in all the species examined.

Areoles are of variable size and polygonal to irregular in outline and are randomly oriented. The frequency, distribution and size of the areole vary in different species or even within the same species. Areoles may contain one or more veinlets and may be without veinlets in the same species. The veinlets are completely absent in the areoles in *Tamarix dioica* and *T. troupii* (figure 1E). The veinlets may be simple (linear or curved) or branched.

The free vein endings are composed of simple tracheidal elements as in the species of *Myricaria* (figure 1G). In species of *Tamarix* the tracheidal elements may be dilated as storage tracheids (Esau 1965) or tracheidal idioblasts (Foster 1956). These tracheidal idioblasts may be solitary or in bunches (figure 1H, I). There is no

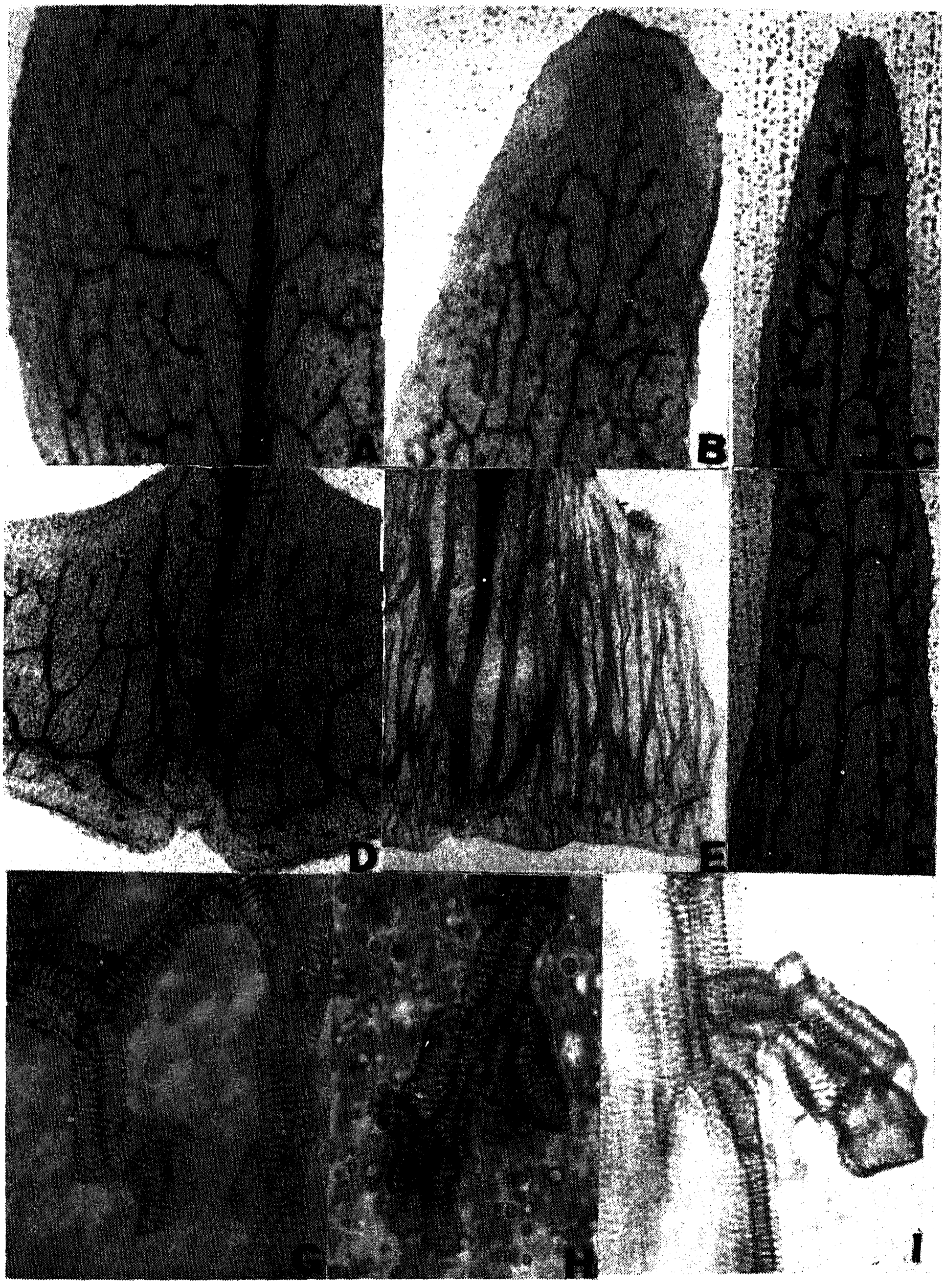

Figure 1. A–F. Portions of cleared leaves showing venation patterns (×60). **A.** *M. germanica.* **B.** *M. alopecuroides* (apical portion). **C.** *T. smyrnensis* (apical portion). **D.** *T. aphylla.* **E.** *T. dioica.* **F.** *T. smyrnensis* (middle portion). **G.** Veinlet endings of *Myricaria germanica* (×320). **H and I.** Tracheidal idioblasts of **(H)** *Tamarix aphylla* (×320) and **(I)** *T. smyrnensis* (×640).

correlation between the size of areole, the number of veins, veinlets and free vein endings. The thickening of tracheids are spiral or annular. A bundle sheath is absent in all the species examined.

4. Discussion

The present investigations show that the size of areoles, the number of vein ending in each areole and structure of vein endings differ not only in different leaves but in different parts of the same leaf. Further, no direct correlation has been observed between the size of areole and the number of vein endings. Therefore, these characters cannot be said to be taxonomically significant, as far as the species of *Tamarix* and *Myricaria* are concerned.

Tracheidal idioblasts have been found either singly or in bunches in *T. aphylla*, *T. gracilis*, *T. smyrnensis* and *T. ramosissima*. These idioblasts are generally attributed to a function of protection and water storage associated with xeromorphism. Their occurrence has been considered to be taxonomically useful by investigators. Varghese (1966) stated that in *Pedicularis* and *Euphorbia* the number of tracheidal idioblasts increased with increase in xerophytic conditions. Kakkar and Paliwal (1972) from their studies on *Euphorbia* species concluded that the occurrence of these elements and xeromorphism are interlinked. The present observation also supports these conclusions.

Acknowledgements

Authors are thankful to Dr A Danin of Israel, Dr H Merker of Sweden and Dr T Pullaiah of Sri Krishnadevaraya University, Anantpur for sending some of the materials used in the present study. One of the authors (AKG) is grateful to the University Grants Commission, New Delhi for the award of a fellowship.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 6, December 1988, pp. 475–482.
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Properties of phosphatases from green alga *Scenedesmus incrassatulus* Bahlin and blue-green alga *Synechococcus aeruginosus* Nag.

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Abstract. Studies on phosphatases (acid and alkaline) in *Synechococcus aeruginosus*, a prokaryotic blue-green alga, and *Scenedesmus incrassatulus*, an eukaryotic green alga under different conditions revealed that the acid phosphatase exhibited maximum activity to pH 4·7 and 37°C in both the algae while alkaline phosphatase displayed greatest activity at 37·5°C and 10 pH in *Synechococcus aeruginosus* and at 10·6 pH and 37·5°C in *Scenedesmus incrassatulus*. The K_m values were found to be 50 and 17 mM for acid phosphatase and 4·2 and 8·3 mM for alkaline phosphatase in blue-green and green algae respectively. The inhibitory effect of metal ions (Cu^{2+}, Fe^{2+}, Hg^{2+}, Zn^{2+}) on acid phosphatase activity is similar in both the algae whereas on alkaline phosphatase, their influence varies. Sodium fluoride and EDTA inhibited both the phosphatases activity with the increased concentrations in both the algae. Benzene hexachloride (50%) retarded these enzyme's activities at higher concentrations in these algae.

Keywords. Acid and alkaline phosphatases; green alga; blue-green alga.

1. Introduction

The existence of non-specific phosphatases in micro-organisms as well as in higher plants is well known and these enzymes supply phosphates from phosphate esters under phosphate deprivation while orthophosphates suppress their activity. The contribution of algae to biogeochemical transformations of soil and enhancement of fertility of soil and aquatic habitats by nitrogen fixation has been well documented (Kumar 1985; Singh 1961; Venkataraman 1966). However, the phytoplanktonic algae of rice fields and aquatic bodies could make use of phosphatases to hydrolyse organic or inorganic phosphates either from stored cellular polyphosphates or the environment as well as aquatic rice fields where farmers very frequently apply the fertilizers and agrochemicals. The measurement of phosphatases activity in algae not only indicates the presence or absence of phosphates in the algal cells but also in the environment including aquatic bodies.

Extensive studies both on intra and extra-cellular acid phosphatases were reported in *Ochromonas danica* (Patni and Aaronson 1974), *Chlorella* sp. (Price 1962), *Euglena* sp. (Blum 1965) and alkaline phosphatase activity in *Rivularia* sp. (Livingstone and Whitton 1984). Sarma and Kanta (1982) evidenced only the activities of acid and alkaline phosphatases in vegetative cells undergoing sporulation of *Anabaena cylindrica*. Earlier studies relating to few properties and localization of phosphatases were mainly confined to *Euglena* sp., *Chlorella* sp. and a filamentous blue-green alga *Rivularia* sp. of aquatic bodies but not of rice fields. In view of lack of adequate information on the properties and kinetics of phosphatases (acid and alkaline) in rice field algae with or without the stress of agrochemicals and fertilizers, comparative studies on the properties of acid and alkaline phosphatases in the eukaryotic colonial alga *Scenedesmus incrassatulus* and the prokaryotic unicellular alga *Synechococcus aeruginosus*, isolated from rice fields, have been undertaken and the observations are reported in this paper. This study is also expected to give an account of eco-physiological aspects of algae in the rice fields.

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2. Materials and methods

2.1 *Materials*

Axenic, clonal population of *S. incrassatulus* and *S. aeruginosus* were raised in a medium worked out by Hughes *et al* (1958) and modified by Allen (1968). The culture as well as experimental methods have been already described (Reddy *et al* 1983). The pH of the basal medium was adjusted to 7·5 for the eukaryotic alga *S. incrassatulus* and 8·5 for the prokaryotic alga *S. aeruginosus* before autoclaving. A 14 day old culture of these algae was employed for enzyme study.

2.2 *Methods*

2.2a *Preparation of phosphatases (acid and alkaline) and their assays*: Harvested algal culture of 14 days old (126×10^6 cells of blue-green alga and 816×10^5 cells of green alga) was washed and suspended in 2 ml ice-cold glass distilled water and then macerated at 0°C in an ice-cold mortar with acid washed sand (700 mg) for about 25 min to obtain aqueous extract. The homogenate was kept in ice-cold for 30 min and centrifuged at 4260 g for 10 min to remove debris and sand particles. The clear supernatant was used for phosphatases (acid and alkaline) assays.

2.2b *Assay for acid and alkaline phosphatases*: The activity of acid and alkaline phosphatases was assayed in duplicate by the methods of Patni and Aaronson (1974) and Bessy *et al* (1946) respectively. The assay mixture (1 ml) for acid phosphatase contained 0·5 ml of 15 mM *p*-nitrophenyl phosphate dissolved in 0·1 M acetate buffer (4·7 pH), 0·2 ml of 0·1 M acetate buffer (4·7 pH) and 0·3 ml of crude enzyme extract; while for alkaline phosphatase, the test solution (1 ml) consisted of 0·5 ml of 15 mM *p*-nitrophenyl phosphate dissolved in 0·1 M carbonate buffer (10·6 pH), 0·2 ml of 0·1 M carbonate buffer (10·6 pH) and 0·3 ml of enzyme extract. The total proteins of crude enzyme extracts of blue-green and green algae were recorded as 63·3 and 30 μg per ml respectively by the method of Lowry *et al* (1951). The assay mixture of acid and alkaline phosphatases were separately incubated for 30 min at 37°C and the reaction was terminated in both these tests by the addition of 5 ml of 0·1 N NaOH. The *p*-nitrophenol released by the acid and alkaline phosphatases was measured at 420 and 405 nm respectively in spectronic-20 spectrophotometer (Bausch and Lomb, USA).

For the measurement of activities of these enzymes, one unit is defined as the quantity of enzyme required to liberate 1 μmol of *p*-nitrophenol per ml per min, under standard conditions. The specific activity was expressed as enzyme units per mg of protein. To study the substrate specificity of acid and alkaline phosphatases of these algae, different phosphate esters (*p*-nitrophenyl phosphate, sodium *β*-glycerophosphate, glucose-1-phosphate, glucose-6-phosphate) were selected and assayed the liberated free inorganic phosphates by the method of Fiske and Subbarow (1925). One unit of enzyme activity on different phosphate esters was defined as the quantity of enzyme required to liberate one μmol of inorganic phosphate per min.

2.2c *Chemicals*: Bovine serum albumin (Sigma Chemical Company, USA), *p*-nitrophenyl phosphate (John Baker Inc., USA) and sodium *β*-glycerophosphate, glucose-1-phosphate, glucose-6-phosphate (Loba-chemie Indoaustranal Co., Bombay) were employed in this study. All the other chemicals used were of highest purity obtained from British Drug House, Bombay.

3. Results and discussion

3.1 *Effect of pH on the phosphatases (acid and alkaline) activity*

Both acid and alkaline phosphatase activities in *S. aeruginosus* and *S. incrassatulus* were assayed using *p*-nitrophenyl phosphate as substrate in 0·1 M glycine-HCl buffer (2·2 − 3·6 pH), 0·1 M acetate buffer (4·2 − 5·6 pH), 0·1 M barbital buffer (6·2 − 8 pH), 0·2 M glycine-NaOH buffer (8·6 pH) and 0·1 M carbonate buffer (9–11 pH). The pH-activity profiles of acid and alkaline phosphatases of these algae is represented in figure 1. It is evident (figure 1) that optimum activity of acid phosphatase was recorded at 4·7 pH in both the algae while the alkaline phosphatase activity was optimum at 10 pH and 10·6 pH in *S. aeruginosus* and *S. incrassatulus* respectively. The above obtained results relating to optimum pH (4·7) for the activity of acid phosphatase of *S. incrassatulus* is slightly different optimum pH range than in *Euglena gracilis* (Blum 1965) and *Ochromonas danica* (Patni and Aaronson 1974) but quite different from the results of *E. gracilis* strain SML-1 (Blum and Buetow 1963). The range of optimum pH (4·7) for acid phosphatase activity was not reported in any blue-green alga, while the optimum activity of alkaline phosphatase ranges also differs in these algae from the earlier reports (Livingstone and Whitton 1984; Aaronson 1971). Thus the pH-activity

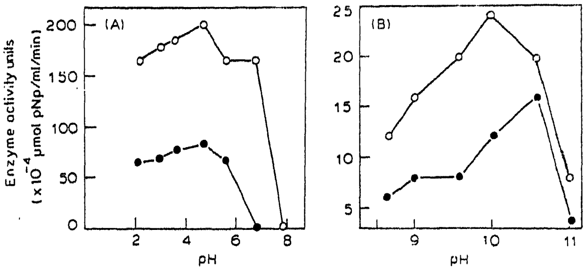


Figure 1. Effect of pH on the activity of acid phosphatase (A) and alkaline phosphatase (B). (○), *S. aeruginosus*; (●), *S. incrassatulus*.

profiles of phosphatases (acid and alkaline) reflect the pH at which the important proton-donating or proton-accepting groups in the enzyme's catalytic sites are in the required state of ionization.

3.2 *Effect of temperature on the phosphatases (acid and alkaline) enzyme*

Effect of temperature on the phosphatases (acid and alkaline) activity in *S. aeruginosus* and *S. incrassatulus* was investigated at 30, 35, 37, 37·5, 45, 55, 60 and 65°C and results recorded are represented (figure 2). It is evident (figure 2A) that the activity of acid phosphatase was optimum at 37°C in both the algae. At 65°C, green algal acid phosphatase became inactive while blue-green algal enzyme was still active. Phosphatase enzyme activity with alkaline buffer was optimum at 37·5°C in both the algae and it became inactive at 60°C (figure 2B). Earlier reports did not mention the temperature tolerance of phosphatases in any algae. The differential activity of phosphatases of green and blue-green algae at different temperatures is probably due to the variation in the inactivation of the associated proteins of the acid and alkaline phosphatases.

3.3 *Enzyme activity at different concentrations of substrate*

The enzyme activities (acid and alkaline) were gradually increased upto 15 mM of *p*-nitrophenyl phosphate in both the algae. In the graphs of double reciprocal plots, substrate concentration was expressed in molars. From the double reciprocal plots (figure 3), the K_m values of acid phosphatase were determined as 50 and 17 mM while for alkaline phosphatase 4·2 and 8·3 mM for blue-green and green algae respectively. Comparative studies indicate the affinity of acid phosphatase towards the substrate in blue-green alga is less when compared to green alga, while in the case of alkaline phosphatase, the affinity towards the substrate is in reverse.

3.4 *Substrate specificity*

Table 1 shows the specificity of acid and alkaline phosphatases towards different phosphate esters in *S. aeruginosus* and *S. incrassatulus*. In both these algae, acid and

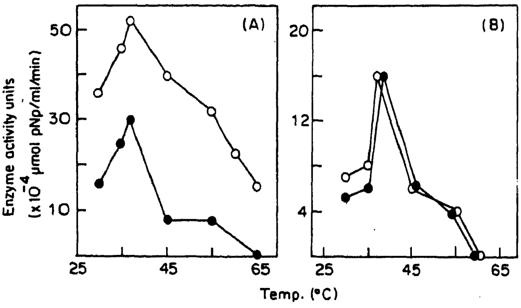


Figure 2. Effect of temperature on acid (**A**) and alkaline (**B**) phosphatases. (○), *S. aeruginosus*; (●), *S. incrassatulus*.

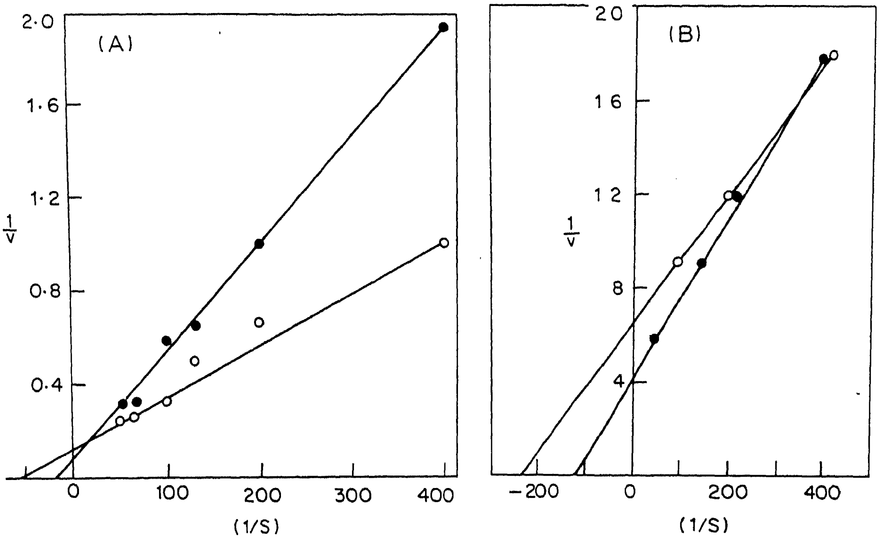


Figure 3. Lineweaver-Burk plots (double reciprocal plots) for acid phosphatase (A) [(●), *S. aeruginosus*; (○), *S. incrassatulus*] and alkaline phosphatase (B) [(○), *S. aeruginosus*; (●), *S. incrassatulus*].

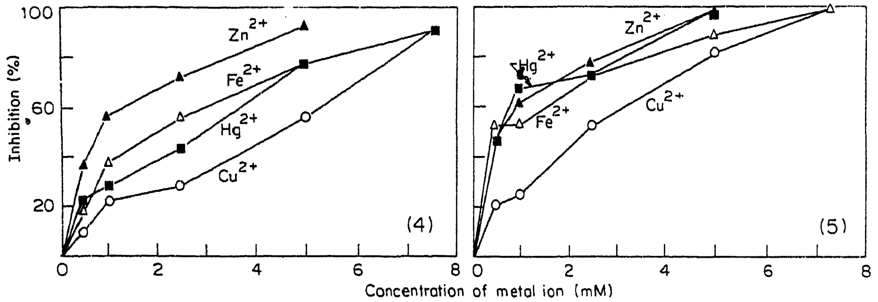
Table 1. Substrate specificity of algal phosphatases [phosphatases activity (μmol phosphate liberated per min) towards *p*-nitrophenol phosphate was taken as 100]. The liberated phosphate per min in alkaline phosphatase is 0·005 μmol (*S. aeruginosus*), 0·003 μmol (*S. incrassatulus*) and in acid phosphatase is 0·004 μmol in both the algae.

	Alkaline phosphatase (relative rate of hydrolysis)		Acid phosphatase (relative rate of hydrolysis)	
Substrate	*S. aeruginosus*	*S. incrassatulus*	*S. incrassatulus*	*S. aeruginosus*
p-Nitrophenyl phosphate	100	100	100	100
Sodium β-glycerophosphate	80	100	207	212
Glucose-1-phosphate	60	67	82	88
Glucose-6-phosphate	69	49	80	81

alkaline phosphatases were found to have high specificity towards sodium β-glycerophosphate as compared to other substrates. None of earlier studies mentioned the results relating to specificities of different substrates.

3.5 *Effect of metal ions* (Zn^{2+}, Fe^{2+}, Hg^{2+}, Cu^{2+}), *sodium fluoride, EDTA and benzene hexachloride 50% on the phosphatases (acid and alkaline) activity*

The activities of acid and alkaline phosphatases of these algae were assayed in different metal ions (figures 4–7) sodium fluoride, EDTA and an insecticide benzene hexachloride (BHC) 50% (table 2). The results represented (figures 4 and 5) indicate that the per cent inhibition of activity of acid phosphatase of these algae increased with the augmented concentrations of metal ions. Among the metal ions, Cu^{2+}



Figures 4 and 5. **4.** Effect of metal ions on acid phosphatase activity in *S. incrassatulus*. (○), Cu^{2+}; (■), Hg^{2+}; (△), Fe; (▲), Zn. **5.** Influence of metal ions on acid phosphatase in *S. aeruginosus*. (▲), Zn; (■), Hg; (△), Fe; (○), Cu.

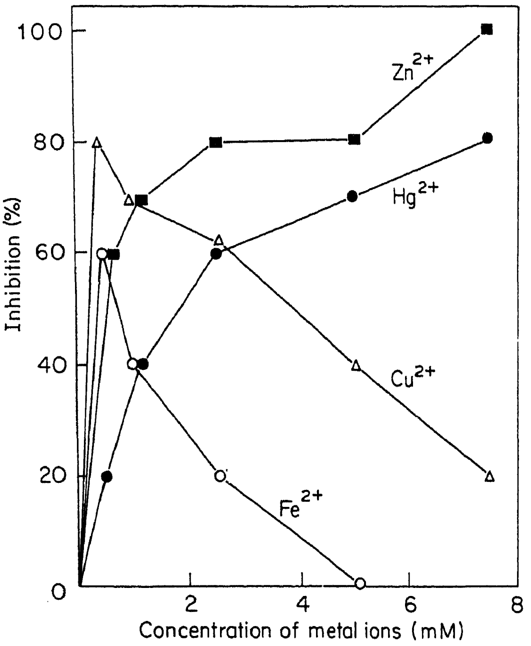


Figure 6. Effect of metal ions on alkaline phosphatase activity in *S. incrassatulus*. (△), Cu; (■), An; (●), Hg; (○), Fe.

appeared to be less inhibitory at low doses as compared to others while with the increased concentrations, Zn^{2+} and Fe^{2+} seemed to be more toxic in both the algae. The per cent inhibition of activity of alkaline phosphatase of these algae was augmented upto 0·5 mM dose of Cu^{2+} and Fe^{2+} and further increase of concentrations, per cent inhibition reduced significantly (figures 6 and 7), Hg^{2+} and Zn^{2+} enhanced the inhibitory activity of alkaline phosphatase in both the algae. It can be inferred that, probably, the low concentrations (0·5 mM) of Cu^{2+} and Fe^{2+} combine with alkaline phosphatase and substrate forms enzyme-metal-substrate complex that led to enhance the inhibition while higher doses of metal ions

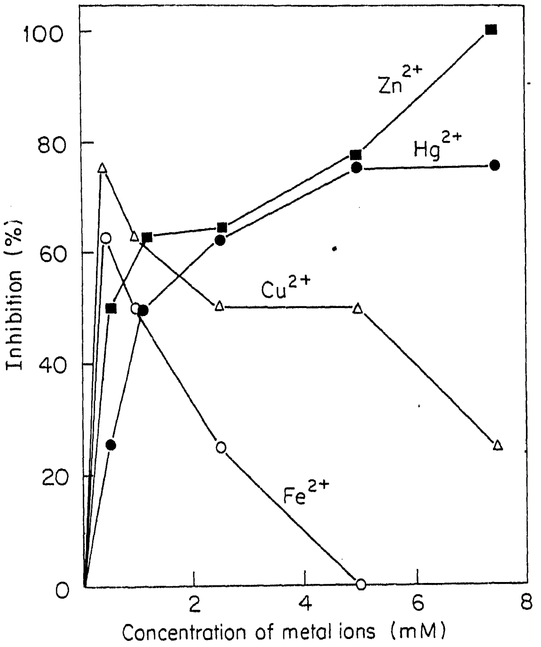


Figure 7. Influence of metal ions on alkaline phosphatase activity in *S. aeruginosus.* (△), Cu; (■), Zn; (○), Fe; (●), Hg.

Table 2. Effect of chemicals on the activity of phosphatases (enzyme specific activity units μmol of *p*-nitrophenol released/min/mg protein).

	Alkaline phosphatase		Acid phosphatase	
Conc. of chemical (mM)	*S. aeruginosus* (specific activity)	*S. incrassatulus* (specific activity)	*S. aeruginosus* (specific activity)	*S. incrassatulus* (specific activity)
Control	0·03	0·05	0·13	0·21
Sodium fluoride				
0·02	0·02(33)	0·02(60)	0·07(46)	0·12(42)
0·03	0·01(66)	0·01(80)	0·07(46)	0·08(61)
0·04	0·01(66)	0·00(100)	0·05(61)	0·03(85)
0·05	0·00(100)	0·00(100)	0·00(100)	0·00(100)
EDTA				
0·02	0·02(33)	0·04(20)	0·13(0)	0·21(0)
0·03	0·01(66)	0·02(60)	0·12(7)	0·16(23)
0·04	0·01(66)	0·01(80)	0·10(23)	0·08(61)
0·05	0·01(66)	0·01(80)	0·07(46)	0·02(90)
BHC 50%				
2·5	0·02(33)	0·02(60)	0·12(7)	0·12(42)
5·0	0·01(66)	0·01(80)	0·10(23)	0·08(61)
7·5	0·01(66)	0·01(80)	0·05(61)	0·02(90)
10·0	0·00(100)	0·00(100)	0·04(69)	0·00(100)

Numbers in parentheses indicate the per cent inhibition.

combine with enzyme that brings out the conformational changes and converting into an active form or the high doses of these metals might be precipitated with alkaline pH range (Mildvan 1970). However, with acid phosphatase of these algae, the reduction of the activity is increased with the enhanced concentration of metal ions attributing to the formation of metalloenzyme substrate complex as mentioned in alkaline phosphatase.

The data in table 2 shows that sodium fluoride (0·05 mM) exerted total inhibition of the activity of acid and alkaline phosphatases in both the algae. Phosphatases (acid and alkaline) activities were inhibited with the enhanced concentrations of EDTA in these algae. Neither of the enzymes (acid and alkaline phosphatases) of these algae could be inhibited significantly at 7·5 mM of BHC (50%) in both the algae while at 10 mM, both the enzymes were completely inhibited except acid phosphatase in *S. aeruginosus*. The inhibitory effect of sodium fluoride on phosphatases of these algae is attributed to the formation of fluoride enzyme complex as reported in *O. danica* (Patni and Aaronson 1974) and *Anabaena* sp. (Reddy and Sarada 1983). The reduction of activity of both phosphatases in these algae with the augmented doses of EDTA indicate that the metal ions might be chelated and make the enzymes inactive. Therefore, it can be concluded that the crude phosphatases require metal ions for their activity. The data (table 2) indicating the effectiveness of BHC on the activity of phosphatases of these algae occurs at very high doses.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 6, December 1988, pp. 483–488.
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N_2-fixation (N_2-ase activity) in *Azospirillum* strains

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MS received 12 April 1988; revised 16 September 1988

Abstract. The nitrogenase activity (N_2-fixation) of *Azospirillum* isolated from the root region of C_4 *Digitaria, Chloris, Cynodon, Dichanthium, Lasiurus* and *Cenchrus* growing in different tracts of Rajasthan, was studied in spot batch cultures on sodium malate medium both under air and 1% oxygen at regular intervals of 24 h for a span of 7 days. The morphological and cultural characteristics of *Azospirillum* strains belonging to *lipoferum* and *brasilense* group (both nir^+ and nir^-) have also been studied. The strains of *lipoferum* group performed better and exhibited more N_2-ase activity on agar surface under 1% O_2 and associated with the roots compared to the strains of *brasilense* group.

Keywords. Nitrogenase activity; *Azospirillum brasilense*; *Azospirillum lipoferum*; C_4 grasses; *Chloris*; *Cenchrus*; *Cynodon*; *Dichanthium*; *Digitaria*; *Lasiurus*; root nitrogenase activity.

1. Introduction

Döbereiner and Day (1976) observed a considerable N_2-ase activity in the rhizosphere of tropical forage grasses. Day *et al* (1975) reported an associative or semisymbiotic nitrogen fixing bacterium, *Spirillum lipoferum* (*Azospirillum brasilense*) from the roots of these grasses. Berggold and Werner (1987) reported a new nitrogen fixing isolate of *Azospirillum lipoferum* from the rhizosphere of *Sorghum bicolar*. Many workers (Smith and Patriquin 1978; Cohen *et al* 1980; Favilli *et al* 1983) surveyed various cereal crops, grasses and dicotyledonous weeds in different countries and concluded that the N_2-ase activity by associative symbiosis in their roots approached that of tropical forage grasses. In the present paper the existence of this system (grass—*Azospirillum* semi-symbiosis) if any in some C_4 grasses of Rajasthan and the performance of best strains of *Azospirillum* are discussed.

2. Materials and methods

C_4 test grasses *Digitaria adscendens, Chloris virgata, Cynodon dactylon, Dichanthium annulatum, Lasiurus sindicus* and *Cenchrus pennisetiformis* were collected from different places of Rajasthan with roots and soil intact. The endophytic bacterium was isolated on (sodium malate) enrichment medium (Döbereiner and Day 1976) for *Azospirillum*, purified and Koch's postulated employing Gibson's (1968) technique. The root section of 30 days old grass seedling was observed for the colonization and establishment by trichloro tetrazolium chloride reduction test (Patriquin and Döbereiner 1978).

The bacterial strains were identified by morphological and cultural characteristics. The cysts formation was observed by malechite green safranin staining technique. After the preliminary screening for nitrogenase activity of all the bacterial strains in obtained from Durgapura Agriculture Farm, Jaipur. It dwindled in 120 h and stopped in 144 h. The pH of the spot changed from 6·8–8 in 7 days.

The strain Asp L-272 isolated from *D. annulatum* from Sikar, did not show N_2-ase activity under a'r but showed activity of 348·979 ± 12·186 nmol C_2H_4·mg protein^{-1}·h^{-1} in 72 h under 1% oxygen. This activity decreased in 96 h and was absent in 120 h. On the other hand, the N_2-ase activity of Asp L-288 isolated from the same host vegetating in Udaipur showed 78·642 ± 1 nmol C_2H_4·mg protein^{-1}·h^{-1} in 96 h under air and 369·368 ± 1·15 nmol C_2H_4·mg protein^{-1}·h^{-1} in 48 h under 1% oxygen. The latter decreased in 72 h and disappeared in 96 h. The pH of the spots in both the cases changed from 7–9·5 in 7 days.

The maximum N_2-ase activity of 35·97 ± 9·25 nmol C_2H_4·mg protein^{-1}·h^{-1} in 72 h under air and 247·95 ± 7·92 nmol C_2H_4·mg protein^{-1}·h^{-1} in 72 h under 1% oxygen at 7·5 pH of the spot, was shown by Asp L-175 isolated from *C. pennisetiformis* collected from Bird Sanctuary, Bharatpur. The activity commenced after 48 h reached climax in 72 h, dwindled thereafter and was absent after 144 h onwards under 1% oxygen while the same appeared after 72 h under air. The pH at the spots changed from 7–8·5 in 7 days.

The strains of *Azospirillum* belonging to both *lipoferum* and *brasilense* groups were further tested for their efficiency to fix nitrogen (nitrogenase activity) in the roots of their respective host plants. Of the 6 test grasses, the maximum nitrogenase activity associated with the roots was 210·50 nmol C_2H_4·g dry root^{-1}·h^{-1} in *C. virgata* inoculated by strain Asp L-4 belonging to *lipoferum* group. This was followed by the activity in *D. annulatum* inoculated by strain Asp L-288 (185·20 nmol C_2H_4·g dry root^{-1}·h^{-1}) and strain Asp L-272 (150·35 nmol C_2H_4·g dry root^{-1}·h^{-1}), *C. pennisetiformis* with strain Asp L-175 (110 nmol C_2H_4·g dry root^{-1}·h^{-1}), *L. sindicus* with strain Asp B-466 (95·10 nmol C_2H_4·g dry root^{-1}·h^{-1}), *D. adscendens* with Asp B-128 (91 nmol C_2H_4·g dry root^{-1}·h^{-1}) and *C. dactylon* with Asp B-132 (51·50 nmol C_2H_4·g dry root^{-1}·h^{-1}) recording the lowest nitrogenase activity in the roots.

It was observed that all the strains of *lipoferum* group exhibited more root associated nitrogenase activity compared to the strains of *brasilense* group.

4. Discussion

Although *Azospirillum* was microaerophilic and was known to show N_2-ase activity under reduced oxygen concentration. Thirty-nine out of 84 strains of *A. lipoferum* and 51 out of 79 strains of *A. brasilense* (both nir$^+$ and nir$^-$) exhibited significant N_2-ase activity on the agar surface under air. Such a behaviour was also observed by Papen and Werner (1980) in *A. brasilense*. Besides, the test organisms were also a little slimy. Further, in the strains of *A. lipoferum* and *A. brasilense* which showed N_2-ase activity both under air and under 1% oxygen, the activity under 1% oxygen was more than that under air. This suggested that the strains had increased confirmational protection besides respiratory protection (Postgate *et al* 1981).

The peak period of N_2-ase activity could be the same both under air and 1% oxygen as in Asp B-466 and Asp L-175, or postponed under 1% oxygen as in Asp B-128 and Asp L-4, or preponed as in Asp B-132 and Asp L-288 (figure 1). The postponement of peak period of N_2-ase activity under 1% oxygen by 24 h was reported by Jagnow (1983) in *A. lipoferum*. In all the efficient strains, pH of agar medium seemed to affect specific N_2-ase activity. In a week long lasting batch cultures, pH of the medium increased slowly and steadily with the alkali production and after pH 7·8 the nitrogenase activity was found to decrease and with further rise in pH of about 8 and above the N_2-ase activity completely ceased (figure 1). Similar observation was also made by Okon *et al* (1976) when they found that at pH 7·8 nitrogenase activity was drastically decreased in *Azospirillum.* Papen and Werner (1982) demonstrated higher rates of N_2 fixation in *A. brasilense* ATCC 29145 increasing the phosphate buffer concentration to 20-fold in sodium malate medium. This increased pH of the medium from 6·8–7·6 during 14 day period suggesting that medium with higher buffer concentration could maintain the nitrogenase activity in *Azospirillum* as compared to a medium with low buffer concentration.

The strain Asp B-132 *C. dactylon* showed oscillating N_2-ase activity (first peak of 122·242 nmol C_2H_4·mg protein^{-1}·h^{-1} in 72 h and second peak of 80·204 nmol C_2H_4·mg protein^{-1}·h^{-1} in 120 h) under air in spot batch culture (figure 1). Similar situation was reported by Papen and Werner (1982) in *A. brasilense* under reduced oxygen concentration. They attributed this to encystation of vegetative cells and their subsequent return to the active vegetative state. In Asp B-132 cysts were not found earlier than 72 h of cultivation. Cysts were detected during 72–96 h cultivation time. At this time most of the cells were immotile and the nitrogenase activity was at its minimum. During the maximum of encystation, no significant increase in growth was observed. After 24 h, the nitrogenase activity increased and the cysts decreased rapidly thereafter. Further all the strains of *Azospirillum* belonging to both *lipoferum* and *brasilense* group when inoculated on their respective hosts exhibited nitrogenase activity (N_2-fixation) associated with the roots. The maximum nitrogenase activity was observed in *C. virgata* inoculated with the strain Asp L-4. The lowest root associated nitrogenase activity was recorded in *C. dactylon* inoculated with the strain Asp B-132. Such low rates of nitrogen fixation by *C. dactylon* has also been reported by Döbereiner and Day (1975) and Ahmad (1979). The performance of strains of *lipoferum* group in terms of root associated nitrogen fixation was better as compared to the strains of *brasilense* group.

Acknowledgement

The authors are grateful to the Department of Science and Technology, New Delhi for financial support.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 6, December 1988, pp. 489–493.
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Response to radiation and *in vitro* growth of two species of *Luzula* with non-localised centromere

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Abstract. *In vitro* growth preceded by gamma-radiation was studied in two species with non-localised centromere, namely *Luzula elegans* and *Luzula pediformis*. Seed germination was affected by radiation doses. Cytological investigation revealed dose dependent chromosomal aberration and spindle disturbances. Chromosome break and fragments were present in *Luzula elegans* but absent in *Luzula pediformis*. The growth of the callus was affected at higher doses in both the species. The radiosensitivity of the two species of *Luzula* with diffuse centromere has been discussed.

Keywords. *Luzula* spp.; non-localised centromere; gamma-irradiation; *in vitro* growth; cytological investigation; radiosensitivity.

1. Introduction

The species of *Luzula* of Juncaceae are characterised by diffuse centromere in the chromosomes. Increase in chromosome number in this genus has principally been affected by fragmentation instead of duplication (Nordenskiöld 1962). Previous studies on DNA estimation have also indicated similar amount of DNA in both diploid and polyploid species (J Sengupta, S Mukherjee and A K Sharma, unpublished results).

The susceptibility and response to culture of the diploid and polyploid species to *in vitro* treatment are not yet known. Moreover instability in chromosome complement often noted *in vitro* has not been studied for diploid and polyploid forms. This is specially significant as fragments arising from such chromosomes have the possibility of survival. Moreover, the radiosensitivity of diploids and such polyploids is yet uninvestigated.

The present study was undertaken with two species of *Luzula* with $2n = 6$ and 12 chromosomes. The species were subjected to gamma-irradiation and the differential behaviour was studied *in vitro* along with control. The objective was to get an understanding of the *in vitro* behaviour of radiation of diploid and polyploid species of *Luzula* with diffuse centromere.

2. Materials and methods

Seeds of *Luzula elegans* and *L. pediformis* obtained from Department of Genetics, Estacao Agronomica National Oeiras, Portugal, were soaked overnight in water and exposed to two different doses of gamma-ray viz. 10 and 20 Gy from a 900 curie ^{60}Co source. After treatment the seeds were washed with 5% Teepol for 10 min followed by several washings in sterile distilled water. These were then surface sterilized with 0·1% $HgCl_2$ for 10–15 min, washed several times with sterile distilled water and inoculated on half-strength basal medium of Murashige and Skoog (1962) solidified with 0·5% agar for germination. Cultures were inoculated at 22 ± 1°C with 55–60% relative humidity under cool, white fluorescent lamp providing 2000 lux light at the culture level for 16 h. A parallel set was maintained in complete darkness. Aseptically germinated seedlings, 1–1·5 cm long, were inoculated on basal medium of Murashige and Skoog (1962) supplemented with 3 mg/l of 2,4-dichlorophenoxyacetic acid (2,4-D) and 300 mg/l of casein hydrolysate (Inomata 1982) solidified with 0·5% agar. Cultures were incubated under similar conditions with 16/8 h light/dark period as mentioned above. Callus was maintained by regular subculturing at an interval of 28–30 days for two months in the same medium.

For studies of chromosome number of callus cells, small pieces of calli were fixed, 4–5 days after subculturing in chilled Carnoy's solution overnight. Squash preparations were made following usual aceto-orcein staining schedule (Sharma and Sharma 1980). Chromosome counts were made for two periods of growth namely, 30 and 60 days. At least 50 ± 10 metaphase plates were counted for each preparation for each species.

3. Results and discussion

3.1 *Germination of seeds*

Seed germination was greatly affected by radiation doses (table 1). With increasing dose, there was a decrease in germination in both species. Moreover, in each species there was a delay in the initiation of germination (table 1). Inhibition of germination or production of abnormal seedlings has been reported earlier in other genera with increasing dose (Bajaj *et al* 1970). However, the germinated seedlings were morphologically identical with the control plants (figure 1).

3.2 *Chromosome study*

In callus tissue of both *L. elegans* and *L. pediformis*, cytological investigations exhibited both chromosomal aberrations and spindle disturbances, the frequency of which were generally dose dependent. Similar findings have been reported in other genera by earlier workers (Sengupta *et al* 1984, 1986; Kar and Sen 1985). Chromosomal aberrations in the present experiment included high percentages of

Table 1. Effect of radiation dose on seed germination in *L. elegans* and *L. pediformis*.

	Minimum days required for seed germination			Percentage of seed germination		
Plant species	Control	10 Gy	20 Gy	Control	10 Gy	20 Gy
L. elegans	21	30	35	16	8	9
L. pediformis	18	30	30	56	45	25

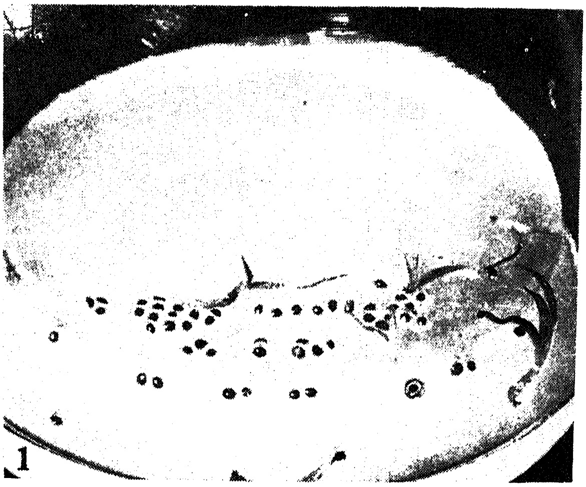


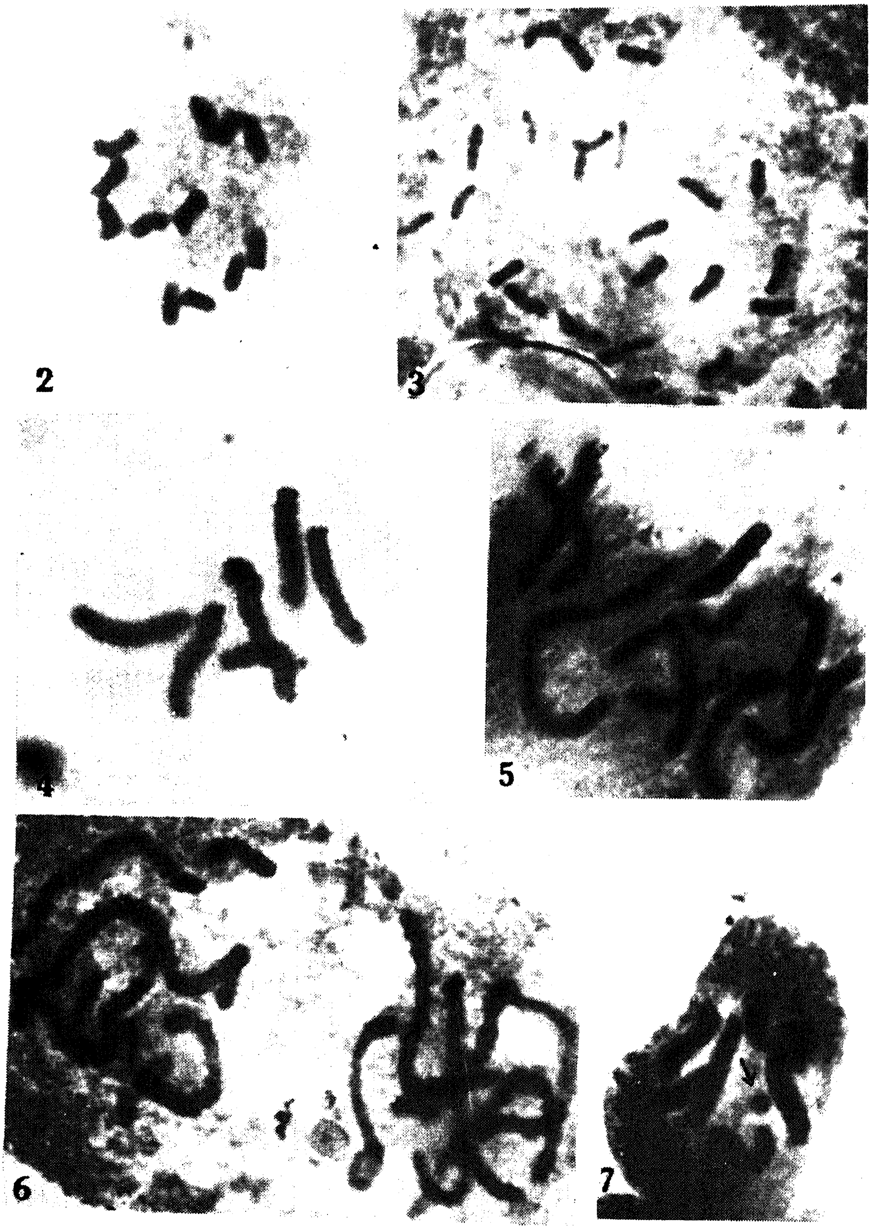
Figure 1. *In vitro* germinated seedlings of *L. elegans*.

metaphase and anaphase stages with fragmentation, clumping, lagging, fusion of chromosome ends, sticky bridge formation as well as disorientation of spindle (figures 2–7).

In *L. elegans*, the percentage of abnormal cells indicated an increase with increasing doses as well as period of culture. This was correlated with a decrease in the frequency of diploid cells under similar condition (table 2).

In *L. pediformis* the same trend was observed in relation to abnormal cells. In this species, however, both control as well as treated cells revealed principally diploid number in different doses as well as after different periods of culture. While chromosome break, fragments and dot chromosomes were most dominant in *L. elegans*, these were absent in *L. pediformis*. Similar fragments and dot chromosomes were observed in radiation induced cells of *L. elegans* by previous workers (Nordenskiöld 1962; Kusanagi 1967). The complete absence of fragments despite diffuse centromeric constitution in any of the sets in *L. pediformis* is of special interest. In species of *Luzula*, increase in chromosome number from $n=3$, is principally through fragmentation of chromosomes (Nordenskiöld 1962; Camara 1951) as fragments are not devoid of centromere. Such a mode of increase in number has also been confirmed through DNA estimation of diploid and polyploid cells. Origin of $2n=12$ in *L. pediformis* is thus through chromosome fragmentation. Once fragmented, the distribution of gene loci in 12 chromosomes has become selectively adapted in the genome. It is not unlikely that any further breakage may destroy the positive balance of the genes and thus lead to non-viability of the cells. Thus the absence of fragments can be accounted for.

Following 20 Gy treatment, the cultures obtained from seeds turned brown after 40 days and could not be studied further. The growth of the callus of two species is thus strongly affected at higher doses of radiation. Similar inhibitory effect of radiation on callus growth has been reported earlier in other plant species (Bajaj *et al* 1970).



Figures 2–7. 2. Diploid metaphase cell of *L. pediformis* (2n = 12) showing fusion of chromosome ends. **3.** Tetraploid metaphase plate of *L. pediformis* with 24 chromosomes. **4.** Diploid metaphase plate of *L. elegans* (2n = 6) from control set. **5.** Tetraploid metaphase plate of *L. elegans* showing 12 chromosomes. **6.** Diploid anaphase cell of *L. elegans* showing disorientation of spindle. **7.** Diploid metaphase cell of *L. elegans* showing six normal chromosomes and a dot-like fragment (→).

Table 2. Frequency of normal and abnormal cells in cultures of *L. elegans* and *L. pediformis* following gamma treatment.

		L. elegans (2n = 6)*			*L. pediformis* (2n = 12)*		
Days in culture	Treatment	No. of diploid cells (%)	No. of > 2n cells (%)	No. of abnormal cells (%)	No. of diploid cells (%)	No. of > 2n cells (%)	No. of abnormal cells (%)
30 days	Control	46·8 ± 2·3 (93·6)	3·2 ± 1·0 (6·4)	2·0 ± 0·98 (4·0)	50·00 ± 0·0 (100)	—	—
	10 Gy	44·0 ± 1·7 (88·0)	6·0 ± 1·2 (12·0)	5·0 ± 1·89 (10·0)	49·45 ± 0·11 (98·9)	0·55 ± 0·23 (1·1)	13·5 ± 1·11 (27)
	20 Gy	37·5 ± 2·8 (75·0)	12·5 ± 1·9 (25·0)	15·6 ± 0·5 (31·2)	48·00 ± 1·1 (96·0)	2·00 ± 0·56 (4·0)	20·45 ± 2·95 (40·9)
60 days	Control	47·0 ± 3·75 (94·0)	3·0 ± 1·1 (6·0)	2·5 ± 1·25 (5·0)	50·00 ± 0·0 (100)	—	—
	10 Gy	30·65 ± 2·5 (61·3)	19·35 ± 1·75 (38·7)	11·25 ± 0·75 (22·5)	41·25 ± 2·05 (82·5)	8·75 ± 1·96 (17·5)	10·0 ± 1·78 (20·0)
	20 Gy	—	—	—	—	—	—

*No. of metaphase cells studied in each species in each set = 50.

Acknowledgements

The authors are grateful to Prof. M Mota, Department of Genetics, Estacao Agronomica National, Oeiras, Portugal, for providing seeds and to the Council of Scientific and Industrial Research, and the Indian National Science Academy, New Delhi for financial assistance.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 6, December 1988, pp. 495–500.
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Transformation of the grain legume *Vigna aconitifolia* Jacq Marechal by *Agrobacterium tumefaciens*, regeneration of shoots

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Abstract. Hypocotyl, stem, leaf and shoot-apex of one-week-old seedlings of *Vigna aconitifolia* were inoculated with the nopaline wild type (T_{37}) of *Agrobacterium tumefaciens* to induce tumours. Hypocotyl segments of 1 cm length from *in vitro* grown plants were also co-cultivated with a bacterial suspension to obtain transformed tissue. Axenic cultures obtained from both the experiments when cultured on basal medium proliferated into callus which differentiated into shoot buds on the same medium after repeated subcultures. The transformed nature of the shoots was confirmed by their failure to produce roots, growth on hormone-free medium and the presence of nopaline and nopaline dehydrogenase.

Keywords. *Agrobacterium tumefaciens*; grain legume; transformation.

1. Introduction

Convincing evidence has accumulated over the last few years indicating that the tumour inducing (Ti) plasmids in *Agrobacterium* are efficient natural gene vectors capable of transferring genes into plant nucleus, where they are stably maintained by covalent integration into the DNA (Nester *et al* 1984). The expression of the T-DNA in the plant cell results in the growth of crowngall tumours, which continue to grow in the absence of exogenous hormones in culture and in the production of the characteristic amino acids, the opines. The Ti plasmid of this natural gene vector has been genetically modified to obtain stable introduction and expression of foreign genes into plant cells. Majority of experiments on transformation so far are restricted to plants belonging to the family Solanaceae (McCormick *et al* 1986; Horsch *et al* 1985).

Among legumes, which comprise important crop species, the majority of experiments on transformation are restricted to the forage legumes such as *Medicago sativa* (Mariotti *et al* 1984; Shahin *et al* 1986), Trifolium species (Webb 1986; White and Greenwood 1987), *Onobrychis viciifolia* (Webb 1986), *Lotus corniculatus* (Webb 1986; Petit *et al* 1987) and *Sesbania rostrata* (Vlachova *et al* 1987). In reports on the grain legumes cowpea (Garcia *et al* 1986) and soybean (Owens and Cress 1985), successful transformation was reported, but no organogenesis could be achieved. Gill and Eapen (1986) and Eapen *et al* (1986) have already established a protocol for plant regeneration in cell, tissue and protoplast cultures of *Vigna aconitifolia*. In the present communication we report the successful transformation of *V. aconitifolia* by wild type Ti plasmid of *Agrobacterium tumefaciens* and its regeneration into shoots.

2. Materials and methods

2.1 *Bacterial strain*

Nopaline strain (T_{37}) of *A. tumefaciens* obtained from National Chemical Laboratory, Poona was maintained on LB medium.

2.2 *Co-cultivation*

Seeds of *V. aconitifolia* were germinated as described before (Eapen *et al* 1986). Hypocotyl pieces of 1 cm length were excised from 6 day old seedlings and incubated for 24 h with 24 h old bacterial suspensions.

2.3 *Tumour induction*

Hypocotyls, stem, leaf and shoot apex of one-week-old seedlings of *V. aconitifolia* growing in pots were inoculated with 24–36 h old cultures of *A. tumefaciens* (T_{37}) after creating a wound with a hypodermic syringe (25 gauge needle). Agar blocks or bacterial suspension in liquid medium were smeared on wounds.

2.4 *In vitro culture of transformed tissue*

Hypocotyl segments along with tumours and co-cultivated hypocotyl segments were rinsed with 70% ethanol for 1 min followed by surface-sterilization with 0·1% mercuric chloride for 5 and 3 min respectively. Aseptic tumours were cut into thin transverse slices and cultured on basal medium containing Murashige and Skoog's (MS, 1962) mineral elements, vitamins after Lin and Staba (1961) along with 3% sucrose. Nutrient medium was solidified with 0·7% agar (SISCO labs, Bombay) after adjusting the pH to 5·8 prior to autoclaving. To prevent the growth of bacteria, carbenicillin (Pyopan, Kosmochem Ltd., Bombay), was added (500 mg/l) to autoclaved medium after Millipore filter-sterilization. For co-cultivated hypocotyls, medium supplemented with 2,4-dichlorophenoxyacetic acid (0·1 mg/l) was used during the first passage and subsequently transferred to MS basal medium. All cultures were grown at 25 ± 2°C under continuous light (1000 lux) and subcultured on fresh medium after 20–30 days.

2.5 *Detection of nopaline and nopaline dehydrogenase*

Nopaline in tumour, callus and regenerated shoots was extracted by homogenising the tissues in equal weight of 70% ethanol. The solution was centrifuged at 12,000 *g* and aliquots applied to Whatman 3 mm paper. Nopaline was detected after separating it by electrophoresis at 400 V for 60 min using formic acid/acetic acid/water (5:15:80 by volume). The paper was dried and stained with a 1:1 solution of 0·02% (w/w) phenanthraquinone in absolute ethanol and 10% (w/v) NaOH in 60% ethanol. Pure nopaline (Sigma Chemical Co., USA) served as the control.

For detection of nopaline dehydrogenase, the method of Otten and Schilperoort (1978) was used. Tumour, callus and regenerated shoots were ground with extraction buffer (0·1 M Tris HCl, 0·5 M sucrose, 0·1% ascorbic acid, 0·1% cysteine HCl, pH 8) centrifuged at 12,000 *g* and supernatant passed through Sephadex G 25 column. The effluent was used for detection of nopaline dehydrogenase. The extract was incubated with 60 mM α-ketoglutarate, 60 mM L-arginine, 16 mM NADH dissolved in 0·2 M phosphate buffer (pH 6·8) for 60 min and 5 μl was spotted, electrophoresis carried out at 400 V for 45 min and stained with 1 vol phenanthrenequinol/ethanol and 1 vol 10% NaOH/60% ethanol. After drying, the spots were visualised under a long wave ultraviolet lamp.

3. Results

3.1 *Response of tumour explant*

Both *A. tumefaciens* grown on semi-solid as well as liquid medium could induce tumours on plants. However, tumours could be produced only on stem and hypocotyl portions, and never on leaf and shoot apex. Age of the seedlings was a critical factor in tumour induction since only young plants till the age day 10 could be made to induce tumours while older plants remained unaffected. Occasionally secondary tumours were also seen on other parts of stem portions of the seedlings. After 2–3 weeks of wounding, the hypocotyl and stem portions showed small protuberances which gradually developed into tumours. These tumours were round and hard in texture. Transverse sections revealed a hard green core encircled with tightly packed green parenchymatous cells. Within a week, *in vitro* cultures of very thin sections of tumours enlarged and developed into a compact callus on basal medium. Sections of hypocotyl or stem of the same size of untransformed tissue failed to grow under identical conditions on basal medium. The compact tumour callus tissue continued to grow vigorously and gradually became friable after each subculture. After two months, in 80% of the callus culture, small and green patches were observed which eventually developed into shoot buds.

3.2 *Response of co-cultivated hypocotyls*

Within a week of culture, sections from co-cultivated hypocotyls started initiating callus on MS medium supplemented with 2,4-dichlorophenoxyacetic acid (0·1 mg/l). Vigorously growing tissue was transferred during second subculture to hormone free medium where it continued to proliferate and did not require hormone for continued growth. About 70% of the callus differentiated shoot buds after two months of culture. Attempts to induce root formation in shoots derived from tumours or after co-cultivation were met with failure, confirming thereby the transformed nature of regenerants.

3.3 *Detection of nopaline and nopaline dehydrogenase*

Crude extract of callus originating from gall tumours, callus and regenerated shoots when tested for nopaline by paper electrophoresis showed the presence of nopaline due to its migration to the same position as standard synthetic nopaline. However, nopaline was not found in untransformed tissue (figure 1). Of the callus cultures tested 80% were found to show the presence of nopaline.

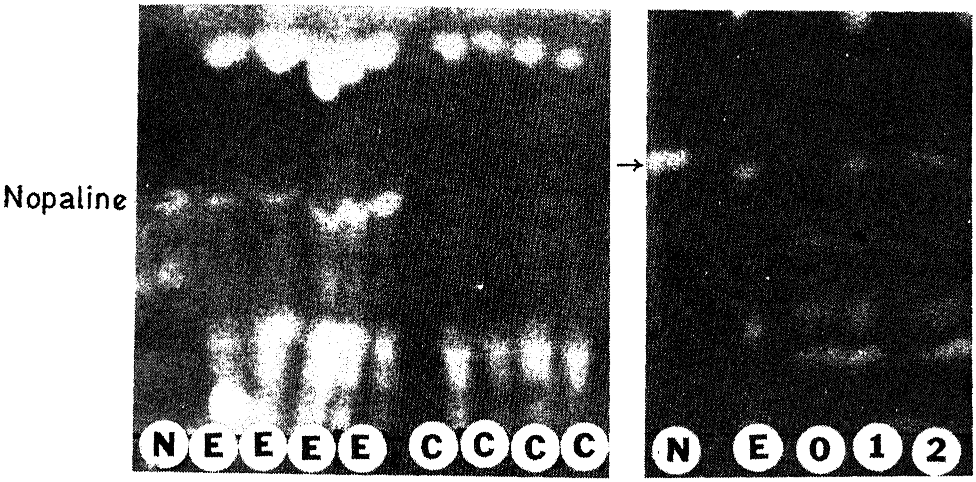
Activity of nopaline dehydrogenase was also tested for the synthesis of nopaline. No traces of endogenous nopaline could be detected after passing through Sephadex G-25 but could be found again after 1 h when effluent from Sephadex column was incubated with incubation mixture (figure 2). The tumour, callus and regenerated shoots showed the presence of nopaline dehydrogenase.

4. Discussion

Since the earlier work on transformation by Lippincott and Lippincott (1967) in legumes, considerable work has followed. Mariotti *et al* (1984) reported successful transformation of cells of a forage legume, *Medicago sativa* to callus and Deak *et al* (1986) obtained shoots from transformed tissue in *M. varia.* Recently Garcia *et al* (1986) have reported transformation of cowpea (*Vigna unguiculata*) cells with an antibiotic resistance gene using a Ti plasmid derived vector. However, they were unable to regenerate whole plants from the transformed callus tissue. Similar results have been obtained in *Glycine max* and *G. soja* (Owens and Cress 1985). We have been able to extend cell transformation studies using an oncogenic type wild strain (T_{37}) of *A. tumefaciens* to the grain legume *V. aconitifolia* using simpler techniques of *in planta* inoculation and co-cultivation with explants. These methods have been used in legumes by earlier workers (Deak *et al* 1986; Garcia *et al* 1986; Mariotti *et al* 1984; Owens and Cress 1985) and also in a few other members of *Solanaceae* namely tobacco, petunia and tomato (Horsch *et al* 1985). Age of the plant was found to be a critical factor for susceptibility to bacterial strain for tumour induction. Similar observation has been made in another grain legume *Glycine max* and *G. soja* (Owens and Cress 1985).

In the present study no difficulty was encountered in culturing tumours *in vitro* whereas in *Medicago sativa*, nopaline tumours could not be successfuly cultured due to necrosis (Mariotti *et al* 1984). Cultures whether derived from tumour explants or co-cultivated hypocotyl segments showed two characteristic features of transformed tissue i.e. hormone-independent growth and the presence of nopaline and nopaline dehydrogenase.

Transformed tissue readily differentiated into shoot buds on basal medium in 80% of cultures. However, shoots did not develop roots; which is a characteristic of tumours induced by wild type *Agrobacterium* strains. Wild type plasmid is known to introduce hormone imbalance and cause problems in regeneration (Akiyoshi *et al* 1983; Schroder *et al* 1983; Nester *et al* 1984; An *et al* 1985). In an earlier study on a forage legume *Medicago sativa*, similar results were observed using the same plasmid (Mariotti *et al* 1984). In mothbean, due to the very small diameter of shootlets, grafting to healthy plants proved difficult. Further attempts are underway in this direction. The relative ease with which cells of *V. aconitifolia* could be transformed by wild type strain of *A. tumefaciens* and its ability to regenerate shoots readily points to the possibilities of using this system for incorporating foreign genes in this species through the use of genetically modified strains. Recently, however chimaeric genes for kanamycin resistance has been transferred to mothbean by direct (Kohler *et al* 1987) and *Agrobacterium* mediated gene transfer



1 2

Figures 1 and 2. **1.** Presence of nopaline in transformed tissue. (N), Standard nopaline; (E), different samples; (C), control samples. **2.** Presence of nopaline dehydrogenase activity. (N), Standard nopaline; (E), extract before passing through Sephadex showing the presence of indigenous nopaline; (O), extract at zero hour showing absence of nopaline after passing through Sephadex; (1–2), shows reoccurrence of nopaline after incubating for 1–2 h in incubation mixture.

(Eapen *et al* 1987) and plants regenerated. Southern blot hybridization had demonstrated the successful integration of bacterial gene into the mothbean genome in these experiments.

Acknowledgements

The authors thank Dr R Mitra for his guidance on paper electrophoresis for detection of nopaline, Dr C R Bhatia for the facilities and Dr M S Chadha for his keen interest in this investigation.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 6, December 1988, pp. 501–505.
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Wind-tunnel estimation of fungi colonizing sorghum seed from field to storage

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MS received 30 May 1988; revised 15 October 1988

Abstract. Microbial loads of sorghum seeds were estimated using a wind tunnel at 3 successive stages; mid ripe, threshing and storage. Very high concentration of field fungi viz., *Curvularia, Drechslera, Fusarium, Phoma* and *Sphacelotheca sorghi* were recorded from seeds at threshing stage, whereas their populations decreased during storage. Storage fungi such as *Penicillium* and *Aspergillus* increased considerably after storage. High concentration of microbes were released even with least agitation.

Keywords. Seed microflora of sorghum; wind tunnel; Andersen sampler.

1. Introduction

Seeds harbour a variety of microorganisms which are responsible for biodeterioration (Thomas 1977), loss of viability (Tripathi 1974; Rati and Ramalingam 1974), mycotoxicosis (Sreenivasamurthy 1977), allergic diseases (Clarke and Madelin 1987) and infection (Gustavo and Mendes 1987).

Seeds receive microbes from air-spora, from other parts of plant, soil, storage structures and infestation by insects (Lacey *et al* 1980). Some may germinate and colonize the growing plant, others may be carried into storage. Which of them develop before or after harvest depends upon the environment. Some fungi may occur only in small numbers, close to the limits of detection before harvest, but the crop may become further contaminated during harvesting, threshing and storage. The microbial load of sorghum seed collected at 3 successive stages; i.e. from the field before harvest when the crop was in mid-ripe stage, from the threshing floor and two months after storage was estimated by wind-tunnel method.

2. Materials and methods

A wind-tunnel similar to the design given by Gregory (1973) was used to estimate the spores released from surface of sorghum seed subjected to varied intensities of agitation. Spores released into wind-tunnel from perforated rotating drum are sampled by an Andersen sampler (Andersen 1958) following Lacey and Dutkiewicz (1976) and rotary drum miniature spore trap (RDMST, Ramalingam 1978). The wind tunnel is used on the basis of a previously planned and tested programme (table 1). A known quantity of sorghum seeds were agitated by rotating the drum approximately at the rate of 0·5 to 2 rotations/s. The air flow was maintained at a constant speed of 1·3 m/s. Samples were taken at different intervals by using Andersen sampler with potato dextrose agar and Czapek Dox agar plates. The

Table 1. A model programme of operation of wind-tunnel used for sampling microflora of sorghum seed.

Interval (min)	Operation	RDMST sampling	Andersen sampling
0–13	No. grain in the drum lab. air sampled	Operated continuously	3–6 min I/CDA 9–12 min II/PDA
13–15	Adding seed to drum	–do–	—
15–20	Seed drum rotation 1 rotation/2 s	–do–	16–17 min III/CDA 19–20 min IV/PDA
20–32	1 rotation/s	–do–	25–36 min V/CDA 30–31 min VI/PDA
32–40	2 rotations/s	–do–	—
40–60	2 rotations/s	–do–	42–44 min VII/CDA 48–50 min VIII/PDA

CDA, Czapek Dox agar; PDA, potato dextrose agar.

sampling period ranged from 0·5 to 3 min. However, air was sampled continuously for 60 min duration using RDMST. Total number of microbes released into the wind-tunnel during the 1 h operation (agitation) was estimated and the effect of agitation on the release of fungi from seed surface was determined. Concentrations of microbes estimated by the Andersen sampler and RDMST are multiplied by the volume of air passed through the wind-tunnel during different episodes and then totalled to arrive at the cumulative total release.

3. Results

Data on the total number of microbes released from 1 kg of sorghum seed at different stages viz., field, threshing and storage, estimated with the help of Andersen sampler are presented in table 2. The data collected with the help of RDMST are given in table 3.

Very high concentrations of microbes were recorded from seeds collected from the threshing floor where *Sphacelotheca sorghi* spores predominate. *Cladosporium*, *Alternaria* and species of *Aspergillus* and *Penicillium* showed continuous increase from field to storage. Fungi such as *Curvularia*, *Drechslera*, *Fusarium*, *Phoma* and *S. sorghi* occurred in high concentrations during threshing stage, whereas they decreased during storage.

Andersen sampler data revealed the existence of very high concentrations of bacteria, which increased with time of storage, whereas actinomycetes showed a decline. RDMST data revealed the presence of very high count of starch grains and fragments of hyphae in addition to spores, where the former predominated in seeds collected from standing crop in the field and the latter in the stored seeds. *Trichothecium roseum* was not recorded in the seed samples collected from the field either by Andersen sampler or by RDMST.

A moderate liberation of microbes from the seed surface was observed even at the least agitation employed in the study (adding the seed to the drum) and a rapid increase with only moderate agitation (1 rotation of seed drum/2 s). The number declined even at higher rates of agitation extending for longer periods. The data on the observed concentrations and estimated number of spores released from the agitated seed at each episode of agitation are plotted against time in figure 1.

Table 2. Total number of microbes released from 1 kg of sorghum seed into wind-tunnel under agitation as estimated by Andersen sampler.

	Seeds collected from		
Colony types	Field	Threshing floor	Storage
Bacteria	2,677·43	5,098·56	6,189·33
Actinomycetes	216·06	59·00	51·72
Alternaria	132·40	166·71	239·59
Aspergillus candidus	—	5·86	11·09
A. flavus	—	—	39·98
A. niger	10·61	13·89	67·70
A. ochraceus	—	—	31·60
A. oryzae	—	—	13·89
Other *Aspergillus* spp.	23·77	109·91	310·43
Cladosporium	871·36	1,516·06	2,239·11
Curvularia	0·71	38·52	20·46
Drechslera	14·38	20·46	13·89
Fusarium	10·27	108·48	28·45
Nigrospora	14·38	—	—
Penicillium	27·31	62·09	662·50
Phoma	57·03	196·09	176·40
Syncephalastrum	—	2·89	6·66
Trichothecium roseum	—	509·71	567·91
Unidentified	120·00	36·04	21·43
Total	4,175·71	7,945·13	10,692·14

Values in thousands.

Table 3. Total number of microbes released from 1 kg of sorghum seed into wind tunnel under agitation as estimated by RDMST.

	Seeds collected from		
Spore types	Field	Threshing floor	Storage
Alternaria	5·55	68·61	70·18
Aspergilli	5·41	16·35	16·80
Cladosporium	228·82	989·91	1,444·63
Curvularia	1·50	16·35	12·27
Drechslera	0·81	21·12	12·27
Epicoccum	—	0·68	2·05
Fusarium	2·50	21·80	8·29
Nigrospora	—	2·73	3·41
Periconia	2·58	0·68	3·41
Puccinia purpurea	1·49	—	—
Sphacelotheca sorghi	25·39	1,730·50	812·02
Trichoconis	4·87	2·73	—
Trichothecium roseum	—	610·47	783·49
Hyphal fragments	16·26	620·05	703·06
Pollen of Poaceae	—	2·04	—
Starch grains	2,043·31	874·84	836·59
Unidentified	3·46	19·76	11·59
Total	2,341·95	4,998·62	4,720·06

Values in lakhs.

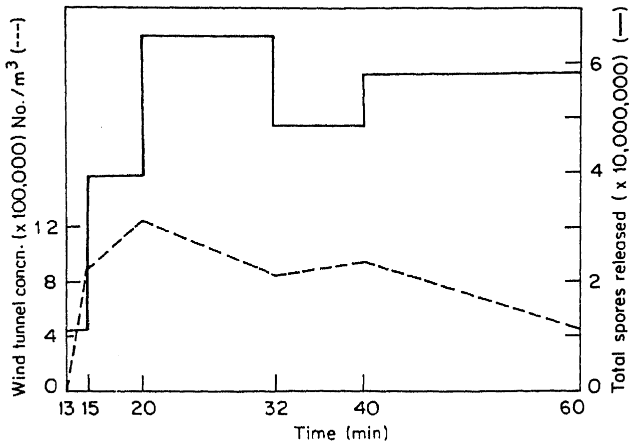

Figure 1. Estimates of fungus spores released from sorghum seed collected at field stage into the wind-tunnel at each episode of agitation.

4. Discussion

Sorghum crop in heading stages often develop head molds which are greater when the earheads are compact and infested by insects (Reddy and Nusrath 1985). In our experiment, ear heads of standing crop developed head molds after the collection of the first batch of seed samples because of their compactness and exposure to long periods of moist rainy weather at the time of harvest. Seeds sampled at threshing stage revealed very high concentrations of *T. roseum*, which however was not recorded on seed collected from field. Probably, spore traps are not as efficient as earheads in receiving inoculum or the fungus might have been received by an insect vector. Very high concentrations of *S. sorghi* during the threshing stage might be due to the rupturing of sori during threshing operation thereby further contaminating the seeds.

Water activity in the stored grain, the intergranular gas composition and temperature of storage are stated to be important factors in the development of microflora in stored grain (Hill and Lacey 1983). Field fungi like *Drechslera*, *Curvularia*, *S. sorghi*, *Phoma*, *Trichoconis*, *Puccinia purpurea* and *Nigrospora* were reduced during storage, whereas storage fungi like species of *Penicillium* and *Aspergillus* were increased during storage as stated by Christensen and Kaufmann (1965).

A large proportion of the microbes on the seed are probably dead as shown by the differences between spore and colony estimates (figure 2). Broken grains and grain dust are stated to predispose seed to microbial invasion and fast deterioration (Lacey *et al* 1980). In such cases the period of safe storage may be reduced. Procedures to reduce such materials before storage may decrease microbial build up in grain and increase storage period. High concentration of starch grains recorded in field collected seeds is probably due to their collection in premature stages and further breakage of grain during separation from ear heads. Seeds collected from threshing floor and storage had a comparatively lesser concentration of starch grains which might be due to their utilization by microbes and insects.

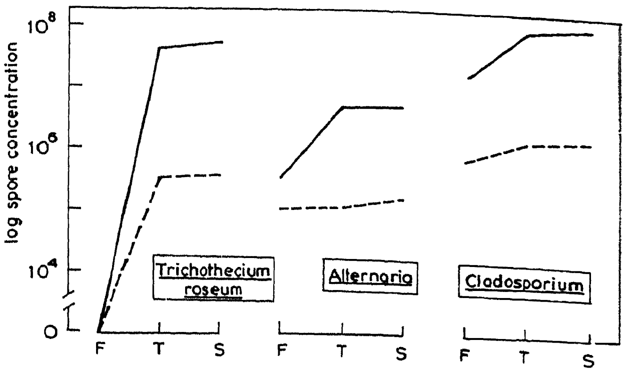

Figure 2. Spore (——) and colony (----) estimates of 3 fungi/kg of sorghum seed at field (F), threshing (T) and storage (S) stages.

Our studies indicate that in addition to grain dust, the starch grains in seed should also be recognised as an important factor in the process of biodeterioration.

Acknowledgement

One of the authors (PS) is grateful to the University Grants Commission, New Delhi for financial assistance.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 6, December 1988, pp. 507–513.
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Growth and foliar variation among provenances of *Eucalyptus camaldulensis* Dehn. grown at Dehradun

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MS received 21 July 1987; revised 24 June 1988

Abstract. Results of provenance trial of *Eucalyptus camaldulensis*, established at New Forest, Dehradun, are discussed. Highly significant differences were observed among the provenances for growth ($P=0{\cdot}001$) and foliar ($P=0{\cdot}01$) characteristics. The provenances of Katherine, NT (S3); Hughendon, Queensland (S7) and Petford, Queensland (S1) from Australia proved best at the site of introduction with mean height 12·97, 13·63 and 12·69 m and mean diameter 10·39, 9·47 and 8·49 cm respectively. Selection of provenances has been advocated on the basis of their comparative performance and within provenance variability. Overall performance of northern and southern populations, having a line of discrimination somewhere between 21° S and 23° S latitude, has been discussed. For the first time statistically significant provenance difference has been explored at stomatal level. Significant negative correlations were obtained between provenance latitude and height ($r=-0{\cdot}64$), diameter ($r=-0{\cdot}73$) and stomata length ($r=0{\cdot}84$). The impact of maximum temperature of the provenance on height and leaf length was observed to be positive and significant ($r=0{\cdot}824$ and 0·710 respectively).

Keywords. Provenance; latitude; coefficient of variation; correlation; northern and southern populations.

1. Introduction

In recent years provenance variation has attracted attention of the forest researchers all over the world. Eucalypts, being a fast growing species and having a wide range of adaptability, have become one of the most studied genera. Studies have been carried out on *Eucalypts camaldulensis* by Bank and Hillis (1969), Hallam and Chambers (1970), Pryor and Byrne (1969), Karschon and Lydia (1971), Burley *et al* (1971), Siddiqui *et al* (1979), Ghosh *et al* (1977) and Venkatesh and Vakshasya (1979).

To assess the performance of different provenances of *E. camaldulensis*, a trial was initiated at New Forest Campus (altitude 640·08 M, latitude 30°20′40″ N, longitude 77°52′12″ E, annual rainfall 216 mm and mean maximum temperature 27·5° C), of the Institute in July 1967. Observations were recorded in 1972 on different growth and foliar parameters.

2. Materials and methods

2.1 *Materials*

Seeds of 12 provenances of *E. camaldulensis*, from 14°25′ S to 34°9′ S, were obtained from Australia (table 1) and seedlings were raised in nursery in polybags. Later these seedlings were outplanted in 1967 in a complete randomised design.

Table 1. Seed origin of *E. camaldulensis*.

Local code No.	Seed lot No.	Origin	Latitude	Max. temp.	No. of plants
S1	6953	Petford, Queensland	17°20′	94·7	48
S2	7116	Goodairs Creek, NT	19°0′	99·8	48
S3	6869	Katherine, NT	14°25′	100·4	24
S4	7080	Newcastle Waters Creek, NT	17°30′	99·4	24
S5	6956	Lachlan River, Forbes, NSW	33°20′	91·9	24
S6	6955	Barrnedman, Wyalong, NSW	34°9′	87·1	24
S7	6949	29 km north of Hughendon, Queensland	20°43′	99·4	12
S8	6957	Macquaris river, Dubho, NSW	32°11′	92·1	12
S9	6960	103 km north of Charleville, Queensland	26°50′	97·6	12
S10	6870	3 km along Quilpic, Eulo, Queensland	28°4′	97·4	12
S11	6788	Bed of Todd River 7 km SE of Alice Spring, NT	23°38′	95·3	12
S12	3052	31 km south of Agnew, West Australia	28°15′	96·7	12

2.2 *Methods*

Measurements were recorded on the trees of 12 provenances of *E. camaldulensis* in 1972.

2.2a *Height and diameter*: Height and diameter at breast height (DBH) of all the available trees were recorded. S12 provenance was not studied due to the large number of damaged trees.

2.2b *Leaf length and leaf width*: Twenty five mature leaves were sampled from the middle of the first persisting branch from 5 different trees of each provenance. Leaves were measured for length, along the midrib and maximum width.

2.2c *Stomata length and width*: For this study 5 leaves were randomly sampled from each of the 5 trees of each provenance. The basal region of the lamina was cut and treated for 12 h in 1:1 ratio of 10% HCl and 10% H_2CrO_4 at room temperature (30° C ± 2). The abaxial epidermal peel separated by this treatment was stained in safranin and mounted in 30% glycerine. One slide was prepared from each leaf. Ten stomata were measured from 5 different microscopic fields under high power (10 × 40) from each epidermal peel prepared. Data on leaf size for 8 provenances and stomata size for 7 provenances could not be taken due to some difficulties.

2.2d *Statistical analysis*: The experiment was laid out in a completely randomised design with varying number of plants per provenance as mentioned in table 1.

Mean (M), standard deviation (SD) and coefficient of variation (CV) were calculated for height and diameter. Standard error also was calculated for the foliar characters as the material was sampled from a population (table 2).

To understand provenance differences emphasis was given to one way analysis of variance and differences between pairs of means for each characteristic were tested by calculating least significant difference (LSD). For height and diameter number of trees per provenance had varied resulting in many values of LSD and hence the bar

Table 2. Statistical constants for height (Ht), diameter (Dia), leaf length (LL), leaf width (LW), stomata length (SL) and stomata width (SW). Values with mean (M), standard deviation (SD), standard error (SE) and coefficient of variation (CV).

Provenance code Latitude		S_3 14°25′	S_1 17°20′	S_4 17°30′	S_2 19°0′	S_7 20°43′	S_{11} 23°38′	S_9 26°50′	S_{10} 28°4′	S_{12} 28°15′	S_8 32°11′	S_5 33°20′	S_6 34°9′
	M	12·97	12·69	10·19	8·07	13·63	5·66	7·83	8·97		6·90	7·76	8·58
Ht	SD	2·547	3·433	1·845	2·139	2·631	2·514	3·985	2·649	—	1·507	3·146	1·923
(M)	CV	19·63	27·05	18·10	26·50	19·30	44·42	50·89	29·53		21·84	40·54	22·41
	M	10·39	8·49	7·52	5·43	9·47	4·15	5·70	6·07		4·40	5·36	5·22
Dia	SD	2·387	3·037	1·586	1·416	2·195	1·046	2·827	2·384	—	0·946	2·489	1·379
(cm)	CV	22·97	35·77	21·09	26·08	23·18	25·21	49·60	39·27		21·51	46·44	26·42
	M	20·10	17·50		17·60		12·10		19·00	13·10		15·10	11·30
	SD	1·093	1·720	—	1·784	—	1·096	—	1·100	0·260	—	1·452	0·710
LL	SE	1·049	0·771		0·800		0·491		0·493	0·117		0·651	0·318
(cm)	CV	5·437	9·828		10·136		9·042		5·789	19·847		9·615	6·255
	M	3·90	5·00		3·00		3·72		3·66	4·86		4·06	4·06
	SD	0·543	0·200	—	0·213	—	0·117	—	0·234	0·166	—	0·244	0·213
LW	SE	0·243	0·089		0·095		0·052		0·104	0·074		0·109	0·095
(cm)	CV	13·923	4·000		7·100		3·145		6·393	3·415		6·009	5·246
	M	25·10	26·61		25·85		26·17		24·47			22·45	22·76
	SD	1·450	1·255	—	2·870	—	0·770	—	1·190	—	—	0·485	0·846
SL	SE	0·650	0·562		1·286		0·345		0·533			0·217	0·379
(μ)	CV	5·776	4·716		11·101		2·942		4·863			2·160	3·717
	M	17·72	18·98		19·99		20·30		18·16			16·90	16·46
	SD	0·682	1·629	—	2·367	—	0·716	—	0·907	—	—	0·571	0·685
SW	SE	0·306	0·726		1·058		0·321		0·406			0·256	0·307
(μ)	CV	3·848	8·582		11·840		3·527		4·994			3·378	4·161

diagrams were drawn. However values of LSD have been mentioned for foliar characteristics (table 3). Coefficient of correlation was calculated between the variables studied and geographical factors and between possible pairs of studied variables (tables 4 and 5). All calculations have been carried out as per the formulae given by Snedecor and Cochran (1967).

3. Result and discussion

3.1 *Growth characteristics*

The measurements recorded on height and diameter showed that the mean height and diameter varied from 5·66 m (S11) to 13·63 m (S7) and from 4·15 cm (S11) to 10·39 cm (S3) respectively. Maximum variability was observed in S9 for height (CV 50·89%) and diameter (CV 49·60%), whereas minimum variability was noticed in the case of S4 for height and diameter (CV 18·10%) and (CV 21·09%) respectively (table 2). This aspect of range and within provenance variability provides the base for provenance selection to raise homogeneous population with acceptable rate of growth. Analysis of variance carried out on height and diameter showed that the differences among the provenances were highly significant. It was observed that provenances S7 (Hughendon), S3 (Katherine) and S1 (Petford) were superior amongst the provenances under test. Bar diagrams drawn on the basis of LSD showed that S1, S3, S4 and S7 are the main contributors for the significant differences between the provenances whereas the performance of rest of the provenances was almost similar thus forming a separate group (table 3).

The natural distribution of *E. camaldulensis* has been grouped into northern and southern populations on the basis of some physiological and morphological features (Pryor and Byrne 1969). The present study also indicated the existence of northern and southern populations on the basis of growth differences. The diameter was found to be a better discriminator with shifting of a single provenance (S2) from the expected position. If a line of delimitation is to be drawn on the basis of bar diagrams obtained as indicated by the result of the present investigation, it will fall somewhere between 21° S and 23° S latitude. In this experiment the northern populations are those north of 21° S latitude and southern populations are those south of 23° S latitude.

The northerly populations were less variable and grew faster than the southern populations. This reflects a better stability and proportional adaptability of low latitude population lending the way for safer selection of some provenance from northern region of Australia. The performance of the best 3 provenances assessed in this study confirms this.

The findings of Siddiqui *et al* (1979) on the comparative performance of *E. camaldulensis* at 6 and 10 years of age were in agreement with the present studies, as the ranking of the provenances at 5 years (the present study) and at 8 years (Gosh *et al* 1977) remained unchanged. This showed that there are possibilities of evaluating the provenances at an early age.

3.2 *Leaf characteristics*

The range of variation in leaf length was observed from 11·30–20·10 cm (S6 and S3)

Table 3. Mean values of the characters studied together with their significance and bar diagram.

													Sig.	LSD
Ht	Provenance	S_7	S_3	S_1	S_4	S_{10}	S_6	S_2	S_9	S_5	S_8	S_{11}		
	Mean	13·63	12·97	12·69	10·10	8·97	8·58	8·07	7·83	7·76	6·90	5·66	**	LSD differs as numbers of trees varied
Dia	Provenance	S_3	S_7	S_1	S_4	S_{10}	S_9	S_2	S_5	S_6	S_8	S_{11}		
	Mean	10·39	9·47	8·49	7·52	6·07	5·70	5·43	5·36	5·22	4·40	4·15		
Bar diagram for Ht		S_7	S_3	S_1	S_4	S_{10}	S_6	S_2	S_9	S_5	S_8	S_{11}		
Bar diagram for dia		S_3	S_7	S_1	S_4	S_{10}	S_9	S_2	S_5	S_6	S_8	S_{11}		
LL	Provenance	S_3	S_{10}	S_2	S_1	S_5	S_{12}	S_{11}	S_6					
	Mean	20·10	19·00	17·60	17·50	15·10	13·10	12·10	11·30				*	1·67
LW	Provenance	S_1	S_{12}	S_5	S_6	S_3	S_{11}	S_{10}	S_2					
	Mean	5·00	4·86	4·06	4·06	3·90	3·72	3·66	3·00				*	0·44
St	Provenance	S_1	S_{11}	S_2	S_3	S_{10}	S_6	S_5						
	Mean	26·61	26·17	25·85	25·10	24·47	22·76	22·45					*	2·37
St W	Provenance	S_{11}	S_2	S_1	S_{10}	S_3	S_5	S_6						
	Mean	20·30	19·99	18·98	18·16	17·72	16·90	16·46					*	1·58

*Significant at 1% of probability. **Significant at 0·1% of probability.

Table 4. Correlation between independent and dependent variables.

	Ht	Dia	Leaf length	Leaf width	St. length	St. width
Latitude	−0·644*	−0·728**	−0·645 NS	0·075 NS	−0·841*	−0·587 NS
Max. temp.	0·824**	0·508 NS	0·710*	−0·296 NS	0·637 NS	0·568 NS

Table 5. Correlation among variables studied.

	Ht	Dia	Leaf length	Leaf width	St. length	St. width
Ht		0·966**	0·695 NS	0·314 NS	0·234 NS	0·239 NS
Dia			0·737*	0·259 NS	0·271 NS	−0·174 NS
Leaf length				−0·210 NS	0·331 NS	0·100 NS
Leaf width					−0·402 NS	0·306 NS
St. length						0·207 NS

*Significant at 5% level of probability. **Significant at 1% level of probability. NS, Not significant.

and width from 3–5 cm (S2 and S1) respectively (table 2). The differences between the provenances were highly significant (table 3). The leaf size was found to be a good indicator for the identification of northern and southern populations. Long and narrow leaves are the distinct features of northern populations whereas southern populations are characterised by shorter and broader leaves. There was much less variation in leaf width in their respective provenances exhibiting more stability in this character as compared to leaf length.

Mean stomata length and width ranged from 22·45 (S5) to 26·65 (S1) and 16·46 (S6) to 20·30 μ (S11). Variability was more in northern forms in comparison to the southern forms. Maximum variation was observed in S2, a northern provenance and minimum in S5, a southern provenance (table 2). Analysis of variance showed highly significant differences between provenances (table 3). The two latitudinally extreme provenances were distinctly different in foliar characters. The leaf and stomata size of the southernmost populations i.e. the New South Wales (S5 and S6), were found to be the smallest.

3.3 *Correlations*

The coefficient of correlation was calculated between the characters studied (dependent variables) and latitude and maximum temperature of the seed source (independent variables) separately. Height, diameter and stomata length were found to be negatively correlated with the latitude ($r = -0{\cdot}64$, $-0{\cdot}73$ and $-0{\cdot}84$ respectively). The association of maximum temperature of the seed source with height and leaf length was positive and significant (table 4). The results of the present study were found in accordance with the findings of Pryor and Byrne (1969) who have reported a trend of height increase with decrease in latitude for 6 months old seedlings. In addition the DBH also showed negative correlation with the latitude of respective provenances. The correlation coefficient was calculated for 15 pairs of variables also. There was significant correlation between height and diameter and diameter and leaf length (table 5).

Burley *et al* (1971) observed the leaf length to be positively correlated with the maximum temperature of seed source rather than with latitude and the reverse for leaf width. In the present study the leaf width was found to be related neither with the latitude nor with the maximum temperature of the seed source. The leaf length was observed on the other hand, to be correlated with the maximum temperature of the seed source but very near to significant level in respect of latitude (table 4). A significant negative correlation was observed between stomata length and latitude of the provenance. On the basis of high values obtained for other characters also which are near to significance level the incorporation of more provenances, in such studies to understand the pattern more precisely, is required.

Acknowledgements

The author gratefully acknowledges the award of a Scholarship by the Ministry of Education, New Delhi. Thanks are due to Mr M S Rawat, for necessary facilities and to Dr M L Kapoor, for his valuable discussion and going through the manuscript.

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Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 6, December 1988, pp. 515–522.
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Seasonal nutrient status of Banjara lake, Hyderabad

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MS received 10 February 1988; revised 8 September 1988

Abstract. Seasonal and diel variations of nutrients viz. nitrate, nitrite, ammonia, soluble reactive phosphorus and silica in Banjara lake (identified by Andhra Pradesh Fisheries Department for pisciculture) during August 1986 to July 1987 were reported. Nitrate, nitrite and ammonia reached highest concentrations in March ($3\cdot76 \pm 1\cdot74$ mg l^{-1}), July (575 ± 77 μg l^{-1}) and April ($404\cdot6 \pm 127$ μg l^{-1}) respectively. However, fish kills through nitrite toxicity was not observed at the reported nitrite concentrations. Ammonia decreased during summer mainly due to utilization by phytoplankton blooms. The highest value of soluble reactive phosphorus was observed in April (956 ± 44 μg l^{-1}) attributed to sewage, domestic effluents and also release from sediments. Silica was at a maximum in October and decreased during diatom blooms in December. Clear pattern of diel variation was not observed for nitrate, nitrite and phosphorus. However, ammonia showed diel variation having higher concentrations at 6 h than 12, 18 and 24 h due to its slower uptake by phytoplankton during nights. Chlorophyll *a* was at a maximum in May (32 mg m^{-3}) at N/P ratio of 5·3 with the dominance of cyanophyceae and chlorophyceae.

Keywords. Nitrogenous nutrients; soluble reactive phosphorus; silica; seasonal changes; Banjara lake.

1. Introduction

Nitrogenous and phosphorus nutrients are abundant in water receiving substantial inputs of sewage, domestic waste and agricultural leachouts. However, smaller amounts may be due to leaching from the sediments and bacterially mediated decomposition of organic matter (Lukatelich and McComb 1986; Ravera *et al* 1986; Uehlinger 1986).

Lakes and fish ponds in and around Hyderabad have been studied to a considerable extent for water quality, plankton and trophic status (Zafar 1964, 1967; Munawar 1970; Seenayya 1971, 1972; Rao 1971; Mohan 1987). Banjara is a small lake with an area of about 0·046 km^2 located in Banjara hills, a posh residential area of Hyderabad (figure 1). Other important lakes in and around Hyderabad are Osman sagar, Himayat sagar (oligotrophic); Hussain sagar (hyper eutrophic); Mir alam and Faukk's sagar (mesotrophic). Banjara has been classified as eutrophic on the basis of total nitrogen (3·07 mg l^{-1}), phosphorus (0·15 mg l^{-1}) nutrients and calcium (94 mg l^{-1}; Johnson 1983). Urbanisation has been growing rapidly during past 3–5 years surrounding the lake for its picturesque scene due to hillocks. The Banjara hotel is located on the south bank of the lake. The lake is used for pisciculture, water source for cattle, recreation area with anthropic activity (washing cloths and vehicles) and it serves as a receptacle for domestic wastes. Andhra Pradesh State Fisheries Department is keen in keeping this lake pollution free since it generates a revenue of about Rs. 60,000–80,000 per year through fish production. Banjara hotel management also concerned about its management and keen to develop the lake as an aesthetic and recreation resort. So far seasonal and diel nutrient status of Banjara lake has not been studied. The only available reports are: mineral accumulation in water hyacinth (Satyakala *et al* 1986) and trophic status (Johnson 1983). Hence, the objective of present study is to investigate the diel variations and to provide information regarding the seasonality of nitrogenous nutrients, phosphorus and silica in the lake and to review the lake trophic status.

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2. Materials and methods

Banjara lake is situated in Hyderabad at an altitude of 1722 ft (latitude 17° 22′ 30″ N; longitude 78° 25′–78° 27′30″ E). It is a shallow lake with surface area of about 0·046 km^2 having an effective length and width of 0·57 and 0·22 km respectively. The total volume of the lake is $0{\cdot}52 \times 10^6\ \text{m}^{-3}$. It has a shoreline of about 1·8 km. The inflow and outflow is seasonal mainly due to rainfall. The dominant macrophyte is *Eichhornia crassipes* and is periodically removed by Banjara hotel management.

Surface water samples were collected in polyurethane cans every month from 3 sites viz. (A) close to road, where anthropic activity is common, (B) near to outlet and (C) near to inlet of the lake at 6, 12, 18 and 24 h from August 1986 to July 1987. Samples were analyzed on the same day. Nutrients were estimated after filtration through GF/C filters. All spectrophotometric absorbency measurements were made on a Hitachi 150–20 model Spectrophotometer. Methods followed for

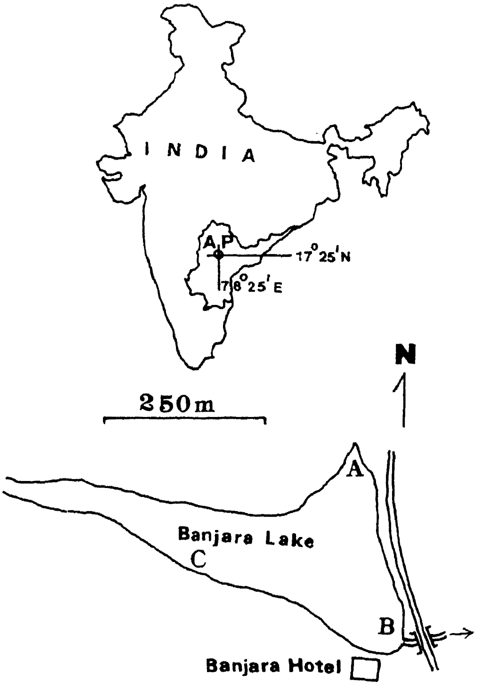

Figure 1. Map showing the location of lake and sampling sites (A, B and C).

analysis of nutrients are ammonia by nesslerization (Michael 1984); nitrate by nitrate-brucine complex, nitrite by sulphonilamide-NED diazotization (Nicholas and Nason 1957); soluble reactive phosphorus (SRP) by molybdenum blue method (APHA 1985; Michael 1984); silica by molybdosilicate method (APHA 1985; Saad and Abbas 1985) and chlorophyll by extraction in 90% acetone (Parsons and Strickland 1965).

3. Results

The synoptic data of the seasonal and diel variations of nutrients at sites A, B and C for the period of one year (August 1986 to July 1987) are presented in figures 2, 3 and table 1.

Nitrate, nitrite and ammonia exhibited diel variation but no clear pattern emerged. However, higher concentration of ammonia were observed at 6 h in all months. Diel and seasonal variations of the 3 forms of nitrogen are shown in figure 2. Nitrite was undetectable during 5 months i.e. August, September, January, February and May. The minimum and maximum amounts of nitrite were 35·8 ± 17·6 (SE) μgl^{-1} in April, 515 ± 132 μgl^{-1} in July respectively. The minimum and maximum nitrate values were 0·56 ± 0·09 mgl^{-1} in November, 3·76 ± 1·4 mgl^{-1} in March respectively. The minimum and maximum values of ammonia were 63 ± 9 μgl^{-1} in January and 404·6 ± 127 μgl^{-1} in April respectively.

There was a seasonal change in nitrogenous nutrients. In March the form in highest concentration was nitrate (3·76 ± 1·4 mg l^{-1}) followed by nitrite

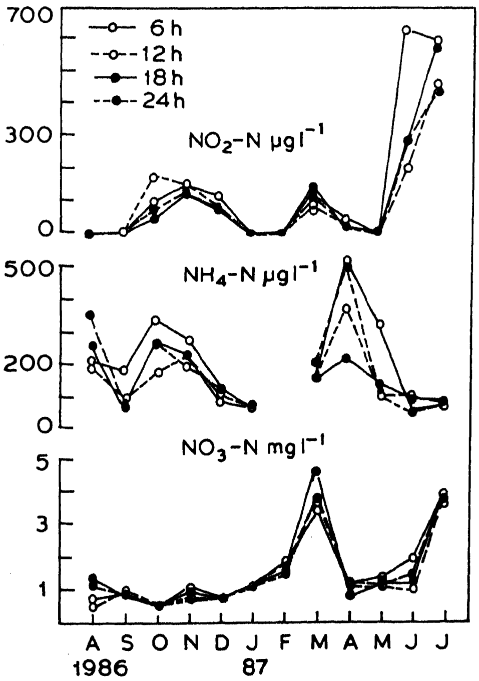

Figure 2. Seasonal and diel variations of nitrate, nitrite and ammonia (average of 3 sites).

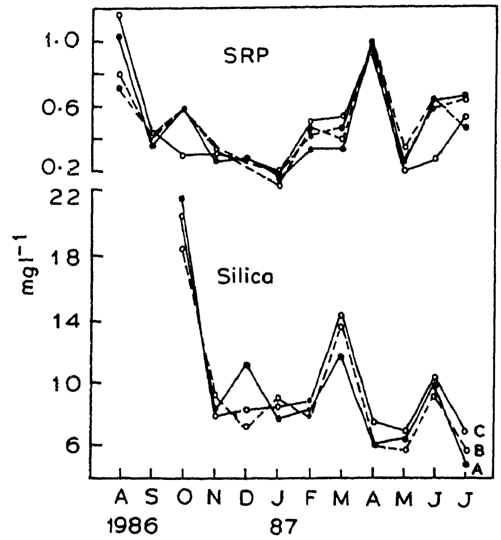

Figure 3. Seasonal variations of soluble reactive phosphorus and silica in sites A, B and C at 12 h.

Table 1. Monthly mean concentrations of ammonia, nitrate, nitrite, SRP and Chl. *a*.

Month	Ammonia (μg l^{-1})	Nitrate (mg l^{-1})	Nitrite (μg l^{-1})	SRP (μg l^{-1})	Chl. *a* (mg m^{-3})
1986					
Aug	255·8 ± 77·8	0·84 ± 0·5	Absent	868·5 ± 167·0	NE
Sep	98·7 + 65·0	0·88 + 0·14	Absent	408·0 ± 64·6	8·6
Oct	260·8 ± 67·0	0·56 ± 0·09	95·2 ± 68·0	545·0 ± 85·0	9·7
Nov	232·0 ± 48·0	0·83 ± 0·27	132·8 ± 25·0	307·0 ± 61·0	NE
Dec	109·5 ± 24·4	0·74 ± 0·14	86·3 ± 28·0	280·0 ± 35·0	19·7
1987					
Jan	63·0 ± 9·0	1·07 ± 0·09	Absent	171·0 ± 53·0	20·2
Feb	NE	1·65 ± 0·48	Absent	480·0 ± 101·0	25·5
Mar	164·4 ± 32·0	3·76 ± 1·4	118·7 ± 47·0	407·0 ± 57·0	30·1
Apr	404·6 ± 127·0	1·04 ± 0·36	35·8 ± 17·6	956·0 ± 44·0	28·1
May	171·0 ± 107·0	1·16 ± 0·3	Absent	247·0 ± 59·0	32·0
Jun	73·8 ± 39·2	1·39 ± 0·46	331·4 ± 214·0	495·0 ± 176·0	27·0
Jul	69·1 ± 37·0	3·84 ± 0·19	515·0 ± 132·0	575·0 ± 77·0	25·8

NE, Not estimated.

(118·7 ± 47 μg l^{-1}) and ammonia (171 ± 107 μg l^{-1}). In April nitrate (1·04 ± 0·36 mg l^{-1}), nitrite (35·8 ± 17·6 μg l^{-1}) were at lower concentrations, and ammonia (404·6 ± 127 μg l^{-1}) reached its highest concentration. In May a slight increase in nitrate (1·16 ± 0·3 mg l^{-1}) and decrease in ammonia (171 ± 107 μg l^{-1}) was observed, however nitrite is absent. Nitrate increased further in June (1·39 ± 0·46 mg l^{-1}) and July (3·84 ± 0·19 mg l^{-1}). In June a sudden rise in nitrite to 331·4 ± 214 μg l^{-1} was observed, and increased further in July (515 ± 132 μg l^{-1}, the highest concentration of the season).

No clear pattern was observed in diel variations of SRP (figure 3). The minimum and maximum values of SRP were $171 \pm 53\ \mu g\,l^{-1}$ in January, $956 \pm 44\ \mu g\,l^{-1}$ in April respectively. In the month of August maximum variations between the 3 sites were observed (mean values of A, B and C were 936 ± 181, 799 ± 98 and $870 \pm 179\ \mu g\,l^{-1}$ respectively).

Site-specific and diel variations were not uniform with regard to silica. The minimum and maximum monthly mean values were $5{\cdot}71 \pm 0{\cdot}9\ mg\,l^{-1}$ in July and $19{\cdot}9 \pm 1{\cdot}33\ mg\,l^{-1}$ in October respectively. Figure 3 represents the values at 12 h in 3 sites. The minimum and maximum values of chlorophyll *a* were in September ($8{\cdot}6\ mg\,m^{-3}$) and in May ($32\ mg\,m^{-3}$) respectively.

4. Discussion

Nitrate available in aquatic systems may either be assimilated by algae and aquatic macrophytes, or transferred to underlying sediments where it undergoes denitrification. Sediments with high concentrations of available carbon remove nitrate faster than sediments with low available carbon (Reddy and Reddy 1987). Nitrification is a rapid irreversible sink for ammonia leading to the formation of nitrite and nitrate (Lipschultz *et al* 1986). This process takes place mainly in the upper centimeters of oxic sediments. It also takes place in the overlying water column at lower rates. Geldermalsen (1985) observed that nitrate showed maximum values in summer and ammonia is produced throughout the year in small amounts and nitrite is produced only in winter and spring.

It has been reported that ammonia is preferred over nitrate by phytoplankton (Horrigon and McCarthy 1982; Miyazaki and Ichimura 1986; Mitamura and Saijo 1986; Reddy *et al* 1987). The observed decrease of ammonia in June and July (figure 2) can be attributed to utilization by phytoplankton. Ammonia was observed in high concentrations at 6 h than 12, 18 and 24 h is because of its uptake variation over diel cycles with slower rates in nights (Furnas *et al* 1986). Toxicity of unionized ammonia to warm-water fishes occurred at $0{\cdot}67\ mg\,l^{-1}$ (72 h, LC-50) for large mouth bass *Micropterus salmoides* (Tomasso and Carmichael 1986). Unionized ammonia never reached such a concentration in Banjara lake during this study. Nitrate showed an increasing trend until March ($3{\cdot}76 \pm 1{\cdot}4\ mg\,l^{-1}$) (table 1), was attributed to a high rate of nitrification. Higher amounts of nitrite in March, and decreased oxygen in March ($3{\cdot}2\ mg\,l^{-1}$) and April ($3{\cdot}68\ mg\,l^{-1}$) (the mean value of other months is $10{\cdot}64\ mgl^{-1}$), support this view. On the other hand denitrification is known to play an important role in fluctuations of nitrate in water (Billen *et al* 1985). A decreased level of nitrate in the summer was explained by Geldermalsen (1985) as follows.

(i) Ammonia is less available because it is consumed more easily by phytoplankton than it is converted by nitrifying bacteria to nitrate.

(ii) The oxygen supply to the upper layer of sediments is for the greater part consumed by enhanced mineralization causing oxygen to penetrate the sediment for only a few millimeters.

(iii) Denitrification in the sediments is enhanced by higher temperature, so that nitrate is converted to nitrogen.

(iv) Nitrate is consumed by the phytoplankton.

High amounts of nitrate in July in this study can probably be explained by the load of sewage due to appreciable rainfall in June (8·2 cm) and July (16·4 cm).

Factors that affect bacteria involved in two step conversion of ammonia to nitrate could cause accumulation of nitrite. Sewage effluents also contain high amounts of nitrite (Lewis and Morris 1986). Nitrite level in June (331·4 ± 21·4 μg l^{-1}) and July (515 ± 132 μg l^{-1}) was mainly due to rainfall caused sewage load and could be toxic to fish (Lewis and Morris 1986). However, no toxicity was observed in terms of fish kill. This may be explained because of the presence of high amounts of chloride and calcium carbonate, which tend to reduce nitrite toxicity (Lewis and Morris 1986).

Ammonia and SRP increased simultaneously due to the bacterial break down of organic matter (Barica 1975). Diel and seasonal variations of nitrogen and phosphorus nutrients were shown in figures 2 and 3. Water temperature may effect the phosphorus cycle in aquatic systems apart from changes in oxygen concentration. Kelderman (1980) noticed that phosphorus was released from sediments from April–September, and adsorbed from October–March. The observed high amounts of SRP in August (868·5 ± 167 μg l^{-1}) and April (956 ± 44 μg l^{-1}) might be mainly due to sewage and domestic waste loading mainly through rainfall (21·8 cm in August, 6·1 cm in March and 0·8 cm in April) and partly due to release from sediments. November, December and January (lowest 171 ± 107 μg l^{-1}) may represent the seasons of phosphorus adsorption (table 1 and figure 3).

The floating macrophyte *Eichhornia crassipes* and the submerged *Elodea densa* are known to remove SRP from waters and are also responsible for changes in phosphorus (Reddy *et al* 1982, 1987; Reddy 1983). However, the role of *Eichhornia* in the dynamics of SRP could not be ascertained in this study due to its periodic removal from the lake.

Silica occurs in natural waters either in solution or as silicate polymers derived from catchment soils or of biogenic origin (from dead diatoms). The soluble reactive silicon is probably the only form of silicon available for diatoms and other phytoplankton (Reynolds 1984). Saad and Abbas (1985) noticed an increase in silicate concentration after rainfall and arrival of flood waters and lower silicate levels in highly saline waters. It is evident that blooming of diatoms decreases soluble silicate (Zafar 1967; Seenayya 1971; Saad and Abbas 1985). Biogenic enhancement of silica in summer was observed due to remineralization of sedimented frustules (Lukatelich and McComb 1986). In this study the highest concentration of silica (19·9 ± 1·33 mg l^{-1}) was observed in October; this decreased to 7·13 ± mg l^{-1} in December (figure 3). The decrease is attributed to diatom blooming in November and December (diatoms in these months were about 50% of the total phytoplankton and they are less than 15% throughout the study period). Seasonal variations may also be due to sediment action, surface runoff and remineralization of sedimented diatoms. Sediment acts as a buffer and releases silica as and when its concentration decreases in the overlying waters (Geldermalsen 1985).

Cyanophyceae were maximum in May and June with more than 50% of the total algae, chlorophyceae in April with 45% of total algae. Chlorophyll *a* was at a maximum in May (32 mg m^{-3}) mainly due to cyanophyceae and partly due to chlorophyceae. The observed chlorophyll values (Chl *a* 8·6–32 mg m^{-3}) are fairly high when compared to the values of 1980 (average total chlorophyll 0·26 $\mathrm{mg\,l^{-1}}$; Johnson 1983) and are comparable to other tropical south Indian eutrophic lakes (Hegde 1985). The highest concentration of chlorophyll *a* was observed at N/P ratio of 5·3 is also nearer to a crude rule of 7:1 N/P ratio supports maximum blooms.

Nitrate is very high when compared to the values of 1980. The maximum nitrate during present study is $3{\cdot}84 \pm 0{\cdot}19\ \mathrm{mg\,l^{-1}}$ (mean annual concentration $1{\cdot}48 \pm 1{\cdot}07\ \mathrm{mg\,l^{-1}}$) and very high when compared to 0·99 $\mathrm{mg\,l^{-1}}$ (average 0·64 $\mathrm{mg\,l^{-1}}$) of 1980 (Johnson 1983). Apart from nitrate, ammonia and nitrite were also present in significantly high amounts (annual means $172{\cdot}9 \pm 101\ \mu\mathrm{g\,l^{-1}}$, $187{\cdot}7 \pm 159\ \mu\mathrm{g\,l^{-1}}$ respectively). Maximum amount of phosphate during present study is $956 \pm 44\ \mu\mathrm{g\,l^{-1}}$. But in 1980 the maximum value (3·4 $\mathrm{mg\,l^{-1}}$) is higher than present study. However, average is 450 $\mu\mathrm{g\,l^{-1}}$ less than current value (annual mean value is $478{\cdot}2 \pm 227{\cdot}5\ \mu\mathrm{g\,l^{-1}}$). Thus, significant increase of nutrients in lake are of considerable interest.

5. Conclusion

This is the first report regarding seasonal and diel variations of nutrients in Banjara lake. Eventhough clear pattern of diel variations were not observed for nitrate; phosphorus and silica; ammonia showed a clear diel variation having higher concentrations at 6 h due to its less uptake during nights. In this lake major source for nutrient loading is sewage, domestic wastes which often enhanced by rainfall. This lake being an interception tank between Osman sagar (Oligotrophic) and Hussain sagar (Hyper eutrophic) a long term study of nutrient dynamics including heavy metals and changes in phytoplankton would be quite useful in taking necessary steps to maintain it pollution free and in identifying biomonitors of aquatic pollution.

Acknowledgements

One of the authors (GNR) is grateful to the University Grants Commission and Council for Scientific and Industrial Research, New Delhi for the award of a fellowship.

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